

Sustainable rearing of crayfish in a recirculating aquaculture
system using the example of *Astacus astacus*

Uli Bernd Seemann

Dissertation zur Erlangung des akademischen Grades eines Doktors der
Naturwissenschaften (Dr. rer. nat.)

Fachbereich Biologie/Chemie

Universität Bremen

August 2019



Cover picture: Uli B. Seemann

DEDICATED TO MY FAMILY

CONTENTS

ABBREVIATIONS

SUMMARY 1

KEY FINDINGS 3

ZUSAMMENFASSUNG 5

OUTLINE OF PUBLICATIONS 9

1. General Introduction and Objectives 11

1.1. Aquaculture in general 11

1.2. Biology of Crayfish 12

1.3. Recirculating aquaculture systems and their potential for sustainable aquaculture 14

1.4. Objectives 16

2. Research Background 21

2.1. Biology of candidate Noble crayfish *Astacus astacus* 21

2.2. Protective measures and economic value 25

2.3. Cultivation and techniques 26

3. Publications 36

Chapter I 36

Seemann, U. B., Lorkowski, K., Slater, M. J., Buchholz, F., & Buck, B. H. (2014). Growth performance of Noble crayfish *Astacus astacus* in recirculating aquaculture systems. *Aquaculture International*, 23(4), 997-1012.

Chapter II	38
Seemann, U. B., Lorkowski, K., Slater, M. J., Buchholz, F., & Buck, B. H. (2017). Feed alternatives for Noble crayfish <i>Astacus astacus</i> based on fatty acid and lipid analyses. <i>Journal of Shellfish Research</i> . 36(2), 519-527.	
Chapter III	50
Seemann, U. B., Lorkowski, K., Schiffer, M., Hörterer, C., Slater, M. J., & Buck, B. H. (2014). Survival of early stripped eggs of the Noble crayfish <i>Astacus astacus</i> and effects of saline solution during artificial incubation. <i>Freshwater Crayfish</i> , 20(1), 1-6.	
Chapter IV	53
Seemann, U. B., Kröncke, N., Slater, M. J., & Buck, B. H. (manuscript). Polyculture potential of Red Nile tilapia (<i>Oreochromis niloticus</i>) and Noble crayfish (<i>Astacus astacus</i> Linnaeus, 1758) in a recirculating aquaculture system. Manuscript.	
Chapter V	75
Seemann, U. B., Hörterer, C. & Buck, B. H. (manuscript). Shelter preference of Noble crayfish <i>Astacus astacus</i> in recirculating aquaculture systems. Manuscript.	
4. Synthesis and Perspectives	88
4.1. Growth performance and feed	88
4.2. Reproduction	89
4.3. System and technique	91
4.4. Polyculture	92
4.5. Conservation, Economics and Sustainability	93
4.6. The Future of Noble Crayfish – A Perspective	94
5. Conclusions	96
Acknowledgements	
Erklärungen	

Abbreviations

1 st	first
2 nd	second
3 rd	third
4 th	fourth
AWI	Alfred-Wegener-Institute, Helmholtz Centre for Polar and Marine Research
AStRa	Astacus Stock Raising
°C	degrees Celsius
CTU	Celsius Temperature Units
DBU	Deutsche Bundesstiftung Umwelt
DNA	deoxyribonucleic acid
FA	fatty acid
kg	kilogram
ICS	indigenous crayfish species
IDeA	Integration of Detrivores in existing Aquaculture systems
IUCN	International Union for Conservation of Nature
KFKS	Koordinationsstelle Flusskrebse Schweiz
MS	manuscript
m ²	square meters
NH ⁴⁺	ammonium
NICS	non-indigenous crayfish species
OIE	The World Organisation for Animal Health
OPS	open pond system
pH	potential hydrogen
PVC	polyvinylchloride
RAS	recirculating aqaculture system
SGR	specific growth rate
ZAF	Centre for Aquaculture Research (Zentrum für Aquakulturforschung)

Summary

Freshwater crayfish play a major role in preservation of natural water bodies and was once found in nearly all freshwater systems. After native crayfish populations in Europe decreased in the late 19th century due to overfishing and an until then unknown disease, crayfish species from North America and concomitant a highly infectious crayfish plague were brought to Europe to revitalize crayfish populations for fishery. Since European crayfish are in contrary to introduced American crayfish not immune to the crayfish plague the rapid decline of indigenous populations started and lasts until now. As a consequence, the native stone crayfish, the Noble crayfish and the White-Clawed are named as threatened species on the Red List by the International Union for Conservation of Nature and Natural Resources.

Since aquaculture production plays an increasingly important role to meet the global demand for aquatic products and expands continuously the idea of an economic production of crayfish came up to finance the conservation of indigenous crayfish in Europe after the motto: protection by use. An enclosed reproduction facility was seen mandatory to minimize the risk of a plague infection and guarantee a safe production.

Therefore, the aim of this study was to examine the potential of a recirculating aquaculture systems for a sustainable culture and reproduction of a European crayfish species. Since the endangered Noble crayfish *Astacus astacus* formerly was a common food source in Europe it presents the economically most interesting native species and was selected for this study. The collaboration with an open pond farm was established to collect and deliver scientific comparative data about crayfish performance under almost natural conditions. Abiotic and biotic parameters like water conditions, growth, density and shelter requirements were recorded. The experiments aimed at establish comparative conditions in a recirculating aquaculture system in accordance with the restrictions of such an artificial system.

Results showed that Noble crayfish can be cultured and reproduced in a recirculating aquaculture system. The reproduction under artificial conditions delivered best results using a five minutes salt bath (26.6% RAS, $P = 0.015$) and excellent hatch rates especially in comparison to open pond systems (100% RAS vs. 20% OPS). Crayfish demands can be answered with e.g. adequate feeds and shelter constructions even though these are not available on the market and have to be manufactured. In addition, stocking densities were

increased to promising numbers (RAS >10 Krebse/m², OPS 1-2 Krebse/m²). Therefore, an economic culture of Noble crayfish in a recirculating aquaculture facility is possible in the long-term but primarily given the moderate growth performance of adult crayfish (RAS 0.78/1.33% SGR) and high investment costs not worthwhile for investors since more profitable and fast-growing species can be produced for the growing market. A polyculture of Noble crayfish and Red Nile tilapia showed the advantage of a co-culture as cleaning intervals are reduced due to finfish bioturbation and feed conversion is increased without a negative impact on growth (ANOVA, $p > 0.05$).

It can be summarized that recirculating aquaculture systems can successfully be used for reproduction and culture of Noble crayfish since they deliver high controllable and secure conditions, of especially for juvenile crayfish. The species requirements can be met even though there is space for improvements in e.g. feed composition and shelter design in regard to technical and economic limitations of recirculating aquaculture systems. The high investment and maintenance costs do not bear good prospects for a mono-culture even for the high value Noble crayfish but a polyculture is within the bounds of economic possibility and offers several advantages. For crayfish conservation actions and further scientific research recirculating aquaculture systems offer excellent conditions especially in the face of the threatening crayfish plague. Taking into account economic aspects of a crayfish culture based on this cost-intensive technique a combination of recirculating aquaculture systems together with more unsecure but convenient open pond systems seems the best compromise about safety and economic arguments to produce native crayfish for restocking purposes. The combination with an open pond system opens the opportunity to not even produce summerlings but adult crayfish at reasonable prices which are necessary for complete restocking actions.

As an outlook, a research facility using both techniques could take over the role of an ark for endangered crayfish species, conserve their different genetic strains to maintain biodiversity and reproduce these strains for restocking purposes according to their local origin. A co-financing through crayfish sells would reduce operating costs and revisit the project idea and motto: protection by use. Finally, such a constitution could be installed as an office of coordination and assistance for crayfish matters to bundle conservation efforts.

Key findings

I Performance of Noble crayfish in recirculating aquaculture and open pond systems

- The overall annual growth in recirculating aquaculture system is higher than in open pond systems.
- One-year old Noble crayfish can be cultured at stocking densities of up to 300 individuals per m².
- Density trials with three-year old crayfish resulted in ten individuals per m² with a survival rate of 100 %.
- The grading of crayfish every six month is recommendable to reduce inhibitory effect of larger dominant individuals on smaller ones. In open pond systems this will only be possible for two and three-year-old crayfish since summerlings and one-year old crayfish are too small to be found.

II Nutrient requirements of Noble crayfish in recirculating aquaculture systems

- Feed and its ingredients are the crucial growth factor for a good growth performance.
- No industrial feed provided a considerable growth in comparison to natural diets.
- The fatty acid composition and amount play an essential role in achieving high growth rates. If not provided in sufficient variety and amount Noble crayfish synthesize fatty acids themselves but leads to lower growth rates.
- Self-manufactured feeds provided better growth rates than OPS crayfish but included among others of 18 fatty acids.

III Reproduction and culture of Noble crayfish in recirculating aquaculture systems

- The culture and reproduction of crayfish in recirculating aquaculture systems is technically and biologically possible.
- Two egg treatment units were developed to allow better egg handling and daily work routines and delivered good hatching results.
- Disturbances e.g. vibrations and light fluctuations illumination can easily interrupt mating process and even lead to loss of eggs while attached to the females.
- No adequate shelters are currently available on the market.

- For juvenile crayfish filter wool is applicable as shelter compartments. It reduces competition for feed, predatory losses though bigger animals, enables an evenly overall growth and offers immediate shelter access from all directions after moulting.
- PVC tubes of different sizes and diameters proved to be appropriate shelters for all crayfish sizes.
- The use of a finfish species proved any additional tank cleaning redundant in polyculture experiments while growth and survival rates were not affected even if in direct contact.

IV Economic potential of Noble crayfish

- Due to economic reasons a mono-culture of Noble crayfish in RAS is not recommendable. Instead, the combination of open pond and recirculating aquaculture systems presents an economic potential: 1st an open pond system for the parental broodstock. 2nd a breeding and 3rd a grow-up system based on recirculating aquaculture techniques and 4th a grow-out open pond system.
- An affordable crayfish feed is mandatory for an economic crayfish culture in RAS
- The use of *Elodea spec.* and mussel meal of *Mytilus edulis* can be used in crayfish production and reduce raw material costs.
- A polyculture with a finfish species can reduce production costs by using idle space and feed and feces.

V Conservation and Sustainability

- Recirculating aquaculture systems offer the safest and secure option in regard to survival and plague risks but at high costs.
- Since Noble crayfish can be culture in recirculating aquaculture systems the secure reproduction of genetic strains is an option.
- Such a system could be used for other endangered crayfish species needs since general crayfish requirements are equivalent and the adaptations to their specific needs are limited.
- Even the secure breeding of plague resistant crayfish in an enclosed environment is possible and might play a major role in crayfish conservation since the crayfish plague can spread without live crayfish.

Zusammenfassung

Europäische Flusskrebse spielen eine große Rolle beim Erhalt der Gewässerqualität und waren in Seen und Flüssen Deutschlands weit verbreitet. Nachdem Ende des 19. Jahrhunderts die heimischen Flusskrebspopulationen in Europa aufgrund von Überfischung und einer bis dahin unbekanntem Flusskrebskrankheit abnahmen, wurden Flusskrebse aus Nordamerika zusammen mit der hochansteckenden Krebspest eingeführt, um die Krebsbestände für die Fischerei aufzustocken. Da Europäische Flusskrebse im Gegensatz zu Amerikanischen Flusskrebse gegenüber der Krebspest nicht immun sind, nehmen die heimische Bestände bis heute rapide ab. Als Konsequenz dieser Entwicklung werden Steinkrebs, Edelkrebse und Dohlenkrebse auf der Roten Liste gefährdeter Arten geführt.

Die stetig zunehmende Bedeutung und Nachfrage von und nach Aquakulturprodukten führte zu der Idee einer nachhaltigen Produktion von heimischen Flusskrebse, um deren Arterhaltung nach dem Motto „Schutz durch Nutzung“ finanzieren und sichern zu können. Eine geschlossene Produktionsanlage wurde als obligatorisch angesehen, um die Gefahr einer Ansteckung zu minimieren und eine wirtschaftliche Produktion zu ermöglichen.

Das Ziel der Studie war daher, das Potential einer geschlossenen Aquakulturkreislaufanlage für eine nachhaltige Kultur und Reproduktion einer europäischen Flusskrebsart festzustellen. Als Kulturspezies wurde der Edelkrebse *Astacus astacus* ausgewählt, da er bereits früher in hohem Maße als Speisekrebse befischt und gehandelt wurde und heute aufgrund seiner Verfügbarkeit als Luxusnahrungsmittel gilt. Die Zusammenarbeit mit einer kleinen Teichanlage zur Krebszucht sollte zur Datenerhebung natürlicher Umweltbedingungen und Wachstumsparameter dienen und eine Vergleichsbasis für die Ergebnisse späteren Experimente im geschlossenen System bieten. Abiotische und biotische Faktoren wie Wasserparameter, Flusskrebswachstum, -dichte und Versteckanforderungen der Tiere wurden untersucht. Diese Erkenntnisse dienten auch dazu, vergleichbare Konditionen im rezirkulierenden Kreislaufsystem zu schaffen, unter Berücksichtigung der Limitierungen und Anforderungen eines solchen Systems.

Die Ergebnisse zeigen, dass eine geschlossene Kreislaufanlage sowohl für die Kultur als auch die Reproduktion des Edelkrebses genutzt werden kann. Die Reproduktion unter künstlichen Bedingungen erzielte die besten Schlupfraten unter Verwendung eines 5-minütigen Salzbad

(26.6% RAS, $P = 0.015$) und sehr gute Überlebensraten insbesondere im Vergleich zum offenen Teichsystem (100% RAS vs. 20% OPS). Die Anforderungen der Krebse können beispielsweise mit entsprechenden Futtermitteln und künstlichen Versteckmöglichkeiten erfüllt werden, auch wenn diese zunächst noch selbst hergestellt werden müssen. Die Besatzdichten konnten im Vergleich zum Teichsystem deutlich erhöht werden und stellen eine wirtschaftliche Kultur des Krebses in Aussicht (RAS >10 Krebse/m², OPS 1-2 Krebse/m²). Bedingt durch die arttypische Wachstumsgeschwindigkeit insbesondere adulter Krebse (0.78/1.33% SGR) und die hohen Investmentkosten einer geschlossenen Kreislaufanlage ist aber eine Monokultur des Edelkrebse für Investoren nicht attraktiv, da sich andere aquatische Arten schneller und damit günstiger für den wachsenden Markt produzieren lassen. Eine Mischkultur aus Edelkrebse und Rotem Nil Tilapia zeigte den Vorteil einer kostenreduzierenden Kulturweise auf, da zusätzliche Reinigungsintervalle durch die fischbedingte Durchmischung der Wassersäule entfallen und die Futtermittelverwertung erhöht wird, ohne das Wachstum der Krebse negativ zu beeinflussen (ANOVA, $p > 0.05$).

Zusammenfassend kann der Einsatz von rezirkulierenden Aquakulturkreislaufanlagen zur Besatzkrebse zucht von Edelkrebsen positiv bewertet werden, da sehr gut kontrollierbare und sichere Bedingungen hergestellt und aufrechterhalten werden können, insbesondere für Jungkrebse. Die Anforderungen werden der Krebse werden erfüllt, lassen aber dennoch Raum für Verbesserungen bei beispielsweise der Futtermittelzusammensetzung und Versteckmöglichkeiten in Bezug auf technische und ökonomische Notwendigkeiten bei Einsatz eines geschlossenen Systems. Die hohen Investment- und Instandhaltungskosten machen selbst für den hochpreisigen Edelkrebse eine Monokultur unwahrscheinlich, wohingegen eine Mischkultur im Bereich der ökonomischen Machbarkeit liegt und Vorteile gegenüber einer Monokultur bietet. Für Arterhaltungsmaßnahmen und weitere Forschungsfragen stellt ein rezirkulierendes Aquakulturkreislaufsystem exzellente Arbeitsbedingungen insbesondere im Hinblick auf die in Gewässern weit verbreitete Krebspest. Betrachtet man die ökonomischen Nachteile bei Nutzung dieser Kreislauftechnologie, so scheint die Kombination dieser sicheren Kulturmöglichkeit mit einem weniger sicherem aber deutlich kostengünstigerem Teichsystem der beste Kompromiss aus ökonomischen und ökologisch nachhaltigen Aspekten, um die Arterhaltung zu gewährleisten und Besatzmaterial zur Verfügung zu stellen. Dadurch ließen neben Jungtieren auch ausgewachsene Tiere zu marktgängigen Preise produzieren, die für eine vollständige und nachhaltige Besatzmaßnahme nötig sind.

Als Ausblick könnte eine Forschungsanlage unter Einsatz beider Kultursysteme die Rolle einer Arche für gefährdete Flusskrebse übernehmen, deren unterschiedliche Genstämme und Diversität erhalten und die Nachzucht dieser Stämme für Besatzmaßnahmen entsprechend ihrer Herkunft ermöglichen. Eine Co-Finanzierung durch Verkauf von Besatzmaterial würde die laufenden Kosten senken und das Motto der Projektidee wieder aufgreifen: Schutz durch Nutzung. Die Einrichtung einer nationalen Koordinationsstelle für Flusskrebse könnte die regionalen Anstrengungen zum Arterhalt bündeln und intensivieren.

Outline of publications

The following overview outlines the three first author publications and two manuscripts included in this PhD thesis. The idea for this study originated from the project AStRa “Sustainable rearing of crayfish in a recirculating aquaculture system using the example of *Astacus astacus*, which was written by myself and Prof. Dr. Buck and funded by the DBU. I developed the general concept of the study with logistic advice and scientific guidance from my supervisors Prof. Dr. Buck and Prof. Dr. Buchholz. All analyses were carried out in the laboratories of the University of Applied Sciences Bremerhaven and the AWI in Bremerhaven, Germany.

- I. U. B. Seemann, K. Lorkowski, M. J. Slater, F. Buchholz, B. H. Buck

Growth performance of Noble crayfish *Astacus astacus* in recirculating aquaculture systems.

I developed the concept and experimental design for this study. Sampling and analysis were conducted by myself with the help of K. Lorkowski. I wrote the manuscript with scientific and editorial advice by Dr. Slater, Prof. Dr. Buchholz and Prof. Dr. Buck. The article is published in *Aquaculture International* (2014).

Aquaculture International 23(4), 997-1012. DOI 10.1007/s10499-014-9859-2

- II. U. B. Seemann, K. Lorkowski, M. J. Slater, F. Buchholz, B. H. Buck

Feed alternatives for Noble crayfish *Astacus astacus* based on fatty acid and lipid analyses

I developed the concept and experimental design for this study. Sampling and analysis were conducted by myself with the help of K. Lorkowski. I wrote the manuscript with scientific and editorial advice by Dr. Slater, Prof. Dr. Buchholz and Prof. Dr. Buck. The article is published in *Journal of Shellfish Research* (2017).

Journal of Shellfish Research 36(2), 519-527. DOI 10.2983/035.036.0223

- III. U. B. Seemann, K. Lorkowski, M. Schiffer, C. Hörterer, M. J. Slater, B. H. Buck

Survival of early stripped eggs of the noble crayfish *Astacus astacus* and effects of saline solution during artificial incubation

I developed the concept and experimental design for this study with the help of K. Lorkowski. Sampling was done by myself and with the help of R. Thiele and G. Jähne. Analysis was

performed by myself. I wrote the manuscript with scientific and editorial advice by Dr. Slater and Prof. Dr. Buck. The article is published in Freshwater Crayfish (2014).

Freshwater Crayfish 20(1), 1-6. DOI 10.5869/fc.2014.v20-1.1

IV. U. B. Seemann, N. Kröncke, M. J. Slater, B. H. Buck

Polyculture potential of red Nile tilapia (*Oreochromis niloticus*) and noble crayfish (*Astacus astacus* Linnaeus, 1758) in a recirculating aquaculture system

I developed the concept and experimental design for this study. Sampling was done by N. Kröncke. Analysis was performed by myself. I wrote the manuscript with scientific and editorial advice by Dr. Slater and Prof. Dr. Buck. The manuscript is ready to be submitted to the Journal of Applied Ichthyology.

V. U. B. Seemann, C. Hörterer, B. H. Buck

Shelter preference of Noble crayfish *Astacus astacus* in recirculating aquaculture systems

I developed the concept and experimental design for this study. Sampling was done by C. Hörterer and myself. Analysis was performed by myself. I wrote the manuscript with scientific and editorial advice by Prof. Dr. Buck. The manuscript is planned to be submitted as a short communication to Freshwater Crayfish.

1. General Introduction and Objectives

1.1. Aquaculture in general

Aquaculture is the farming of owned aquatic organisms, such as plants, fish, shellfish and some other invertebrates under fresh and salt water conditions in circumscribed areas and enhancing production yields by e.g. feeding (Boyd, A. McNevin, 2014; Taylor, Kluger, 2016). Several sub forms of aquaculture and methods exist like mariculture and integrated multi-trophic aquaculture (IMTA), which refers to environmental conditions or methods for better resource handling and sustainability (Chopin, 2013; Ridler, Wowchuk, Robinson, Barrington, Chopin, Robinson, Page, Reid, Szemerda, Sewuster, Boyne-Travis, 2007). While mariculture represents farming in marine environments an IMTA is a specific form of polyculture and uses species from different trophic levels, e.g. for a highly efficient resource use (Phillips, 2018; Taylor, Kluger, 2016). Nowadays well known is a farming method, commonly called aquaponics, which bases on an IMTA concept, but includes fresh water plant species (Lennard, Goddek, 2019; Palm, Knaus, Appelbaum, Strauch, Kotzen, 2019).

Since fisheries production stagnates because of e.g. modern and highly efficient trawler fleets causing an overfishing there is still an increasing demand for aquatic products due to a vast increasing world

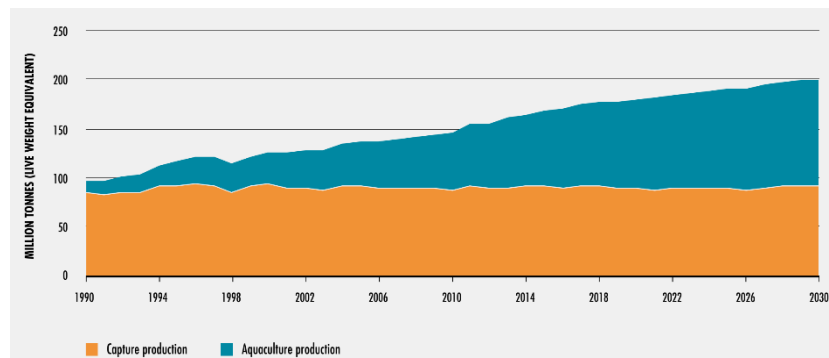


Figure 1 World Capture Fisheries and Aquaculture production, 1990-2030. Source: FAO (2018).

population (Telesetsky, 2017). Since worldwide fish production and consumption is still increasing and fisheries yields stagnate due to overfishing and climate change. Today, the global aquaculture production exceeds already fisheries wild capture and delivers more than 50% of aquatic organisms for human consumption (FAO, 2012). Fisheries production is expected to reach 91 million tonnes resembling production of 2016 meaning only aquaculture production and new technical approaches can satisfy this demand to reach the assumed 200 million tonnes in 2030 (Fig. 1) (Bong Chang, Lee, 2018; FAO, 2018). In 2030, the major growth of fish production is expected to originate with up to 60 percent from aquaculture with in total 109 million tonnes (Bank, 2013; FAO, 2018).

1.2. Biology of Crayfish

Crayfish are known as a key species in the ecosystem and play a dominant role in the food web especially as detritus converter (Maiwald, 2007). As omnivores they feed on insects, worms, mussels, plants and old or ill fish while fish, birds and mammals feed on crayfish (Füreder, 2009; Hager, 2003; Kozák, Duris, Petrusek, Buric, Horka, Kouba, Kozubikova-Balcarova, Policar, 2015). Most of these species are night active and dig holes into the ground or build shelters for coverage during the day. These interventions can shape their environment drastically (LAVES, 2011). Crayfish and especially the Noble crayfish (*Astacus astacus*) played a major role for fisheries and were traded as live feed and for stocking purposes over long distances (Cukerzis, 1988; Füreder, 2009; Keller, 2017). Today only few populations of native species remain in Europe (Edsman, Füreder, Gherardi, Souty-Grosset, 2010; Füreder, 2009; Kozák, Füreder, Kouba, Reynolds, Souty-Grosset, 2011). Even though long time translocation by human trade mixed populations and watered different genetic strains new molecular analyses allow the identification of these remaining populations and still reveal these genetic strains and their origin (Schrimpf, Schulz, Theissing, Pârvulescu, Schulz, 2011). The protection of indigenous crayfish species (ICS) including Noble crayfish and its genetic resources is targeted by different federal programs (Chucholl, 2012; Chucholl, Dümpelmann, 2017; LAVES, 2011; Möllers, 2013; Peer, Pfeiffer, 2008; Vaeßen, Groß, Nowak, 2016).

Species systematics, transfer and diseases

Three families of crayfish exist. Two are related to the Northern Hemisphere (Astacidae and Cambaridae) and one to the Southern Hemisphere (Parastacidae) (Reynolds, Souty-Grosset, 2011). In Europe six crayfish species are still existent, three of the Astacidae family, such as *Astacus astacus*, and three of the Austropotamobius family, such as *Austropotamobius torrentium* (Kozák, Duris, Petrusek, Buric, Horka, Kouba, Kozubikova-Balcarova, Policar, 2015). Over 100 crayfish species of the Parastacidae are known in Australia, e.g. *Cherax quadricarinatus* and *Cherax destructor* presenting two of the largest crayfish species. In New Zealand, only two endemic species were found (Reynolds, Souty-Grosset, 2011). Crayfish from these two countries and Europe are threatened by the crayfish plague (Füreder, 2009; Reynolds, Souty-Grosset, 2011). In America more than 405 species are known (Reynolds, Souty-Grosset, 2011). The most important ones are the Spiny Cheek crayfish (*Orconectes limosus*) and the Signal crayfish (*Pacifastacus leniusculus*). Those two play a major role as invasive species and plague carrier in Europe (Holdich, Reynolds, Souty-Grosset, Sibley, 2010).

After crayfish populations in Europe decreased in the late 19th century due to overfishing and intervention into the environment, crayfish species (e.g. *Orconectes limosus*) from North America and concomitant the highly infectious crayfish plague (*Aphanomyces astaci*) were brought to Europe to revitalize crayfish populations for fishery (Füreder, 2009; Holdich, Reynolds, Souty-Grosset, Sibley, 2010; Reynolds, Souty-Grosset, 2011). Four strains of the fungus are known and two of them recorded in Germany while all four already in Europe (Oidtmann, Cerenius, Schmid, Hoffmann, Söderhäll, 1999; OIE, 2019). The fungus leads to nearly 100% extinction of a native population while non-indigenous species (NICS) are immune or at least resistant (Hager, 2003; Holdich, Reynolds, Souty-Grosset, Sibley, 2009; Jussila, Makkonen, Vainikka, Kortet, Kokko, 2011a; Viljamaa-Dirks, Heinikainen, Nieminen, Vennerstrom, Pelkonen, 2011). Together with this fungal disease, the invasive species reduce native populations in size and numbers dramatically while more and more crayfish were imported (Holdich, Reynolds, Souty-Grosset, Sibley, 2010; Peay, Füreder, 2011). Fishkeeping and the concomitant problem with fish release made way for further American crayfish species: Calico crayfish (*Orconectes immunis*), Louisiana crayfish (*Procambarus clarkii*) and the parthenogenetic Marbled crayfish (*Procambarus fallax* f. *virginalis*) (Kozák, Duris, Petrusek, Buric, Horka, Kouba, Kozubikova-Balcarova, Policar, 2015). In Germany, the Spiny Cheek crayfish is now the dominant species, followed by the Signal crayfish, which was introduced to European countries in 1970 (Füreder, 2009; Holdich, Reynolds, Souty-Grosset, Sibley, 2010). These changes have been subsidized by different environmental changes which have had a direct and negative impact on native European crayfish. The Straightening of riverbanks and a subsequent higher stream velocity, agriculture (over)fertilization and climate warming expose ICS to higher stress than NICS while some NICS like *Procambarus clarkii* even benefit from these changes (Holdich, Reynolds, Souty-Grosset, Sibley, 2010; Reynolds, Souty-Grosset, 2011). These stress factors in turn favor viral, bacterial and fungal diseases like White Spot Syndrome disease and Burn Spot disease which increase the decline of ICS populations (Behringer, 2012; Edgerton, Evans, Stephens, Overstreet, 2002; Walther, 2008).

As a consequence, the native stone crayfish (*Austropotamobius torrentium*) is classified as highly endangered, the Noble crayfish (*Astacus astacus*) and the White-Clawed crayfish (*Austropotamobius pallipes*) as critically endangered (IUCN, 2019; Peay, Füreder, 2011). The native Galician crayfish (*Astacus leptodactylus*) from East Europe was introduced as well for compensation together with Spiny Cheek crayfish, but is also under threat of the crayfish

plague. This development led to a high interest in environment conservation actions supported with funding opportunities. While only few small crayfish farms for restocking purposes still exist in Germany, bigger Galician crayfish farms are still running in e.g. Bulgaria and produce several tons per year for consumption. However, the plague is already known to move even faster via e.g. plague afflicted exuvia shells by drifting as it was already spotted in Danube Delta (Schrimpf, Pârvulescu, Copilas-Ciocianu, Petrusek, Schulz, 2012). Thus, it is only a matter of time till the plague and American crayfish get even in secluded areas by one way or the other and lead to a collapse of crayfish farms and populations.

1.3. Recirculating aquaculture systems and their potential for sustainable aquaculture

The technically most demanding farming method is the use of recirculated aquaculture systems (RAS). Driven by the need for a more efficient water (re-)use in intensive recirculating systems, especially in arid regions, research was done to adapt and upscale technologies from already existing domestic wastewater

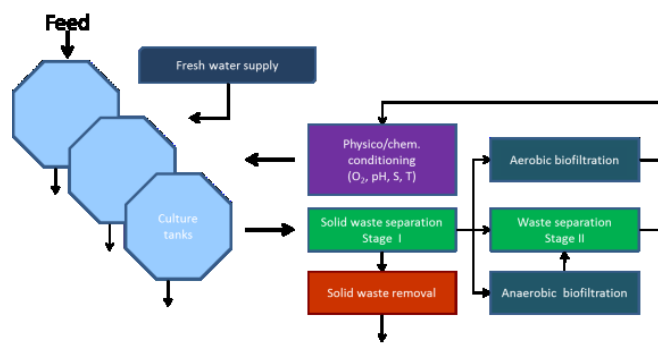


Figure 2 General concept of a RAS. Source: A. Bischoff-Lang.

treatments (Espinal, Matulić, 2019; Murray, Bostock, Fletcher, 2014). Relatively new at least concerning industrial dimensions, these enclosed systems offer constant, secure and adaptable culture conditions for reliable production as they are independent from environmental conditions (Bregnballe, 2015; Dalsgaard, Lund, Thorarinsdottir, Drengstig, Arvonen, Pedersen, 2013; Espinal, Matulić, 2019). The surrounding structure is in general a new built hall with excellent thermal insulation and a ventilation system with heat recovery (Murray, Bostock, Fletcher, 2018). Per definition the water exchange rate must be under ten percent per day which means that almost every home aquarium is already a RAS even though a very small one. Mechanical filters are used to remove solids and biofiltration and denitrification units for ammonia reduction and removal which is toxic for fish at certain levels. Ozone is often used together with skimmers to remove protein via foam fractionation and to hold the bacteria load at a low level (Hutchinson, Mathew, O'Sullivan, Casement, Clarke, 2004; Murray, Bostock, Fletcher, 2014; Sander, 1998). Instead of ozone ultraviolet light can be used as bacteria treatment as well but with less efficiency (Bregnballe, 2015; Hutchinson, Mathew, O'Sullivan, Casement, Clarke, 2004). Aeration is utilized at higher stocking densities to supply

fish with oxygen while at high densities or in critical situations oxygen is directly supplied (Hutchinson, Mathew, O'Sullivan, Casement, Clarke, 2004). All technical systems can be monitored and controlled via measuring and control devices including media and mobile systems for remote access (Bregnballe, 2015; Hutchinson, Mathew, O'Sullivan, Casement, Clarke, 2004; Murray, Bostock, Fletcher, 2018). The prediction e.g. fish growth or nitrogen excretion in combination with models of waste water treatment processes is possible as well as the simulation of effects on RAS in advance (Wik, Lindén, 2004).

1.4. Objectives

By excluding the risk of infection and offering stable and controlled growing conditions, RAS may offer an opportunity for a stable and economically viable production of *A. astacus* for the food market or for restocking programs, compared to open pond systems (OPS). In addition, such a culture would make long term studies possible e.g. for plague resistant breeding programs. A project to evaluate the suitability of an enclosed aquaculture facility was set up. A RAS provides several advantages such as biosecurity aspects, control and monitoring over all abiotic and biotic parameters and independent water treatment, especially for research purposes. Funded by the Deutsche Bundesstiftung Umwelt (DBU) the project (no. AZ28879) addressed several aspects to give conclusions about production feasibility of Noble crayfish in RAS. These aspects dealt with system design, growth performance, feed, reproduction, polyculture concepts and economics. The following objectives and topics were selected and targeted with the listed manuscripts and publications:

- What are the general RAS suitability parameters and requirements for Noble crayfish (tank design, water currents for faeces cleaning, temperature, light)?
- Which growth performance of Noble crayfish in RAS vs. OPS can be achieved?
- Which feeding and feed compositions for Noble crayfish in RAS is necessary and suitable (energy, protein and fat content, fatty acid composition)?
- Can industrial fish feeds fulfill crayfish needs?
- How efficient is Noble crayfish breeding in RAS vs. OPS?
- Is Noble crayfish suitable for polyculture?
- What kind of shelter is applicable for crayfish in RAS?
- What are the opportunities and solutions for crayfish culture and conservation?

The results of these objectives will give insight about the opportunities of RAS for Noble crayfish culture.

References

- Bank, T.W., 2013. Fish to 2030 Prospects for Fisheries and Aquaculture. Agriculture and Environmental Services Discussion Washington.
- Behringer, D.C., 2012. Diseases of wild and cultered juvenile crustaceans. Journal of Invertebrate Pathology. 110, 225-233.
- Bong Chang, J., Lee, Y., 2018. The effects of technological development on fisheries production. Fisheries Science. 85.
- Boyd, C., A. McNevin, A., 2014. An Overview of Aquaculture. 1-20.
- Bregnballe, J., 2015. A Guide to Recirculating Aquaculture - An introduction to the new environmentally friendly and highly productive closed fish farming systems. FAO; EUROFISH, Copenhagen.
- Chopin, T., 2013. Aquaculture Aquaculture , Integrated Multi-trophic (IMTA) aquaculture integrated multi-trophic (IMTA). 184-205.
- Chucholl, C., 2012. Notorisch gefährdet: Probleme, Strategien und Perspektiven im Artenschutz für Edelkrebse, SVK-Fischereitagung, Fulda, pp. 18.
- Chucholl, C., Dümpelmann, C., 2017. Sondergutachten 2017: Erstellung einer Expertise zu Krebsperren und alternativen Schutzmaßnahmen für den Steinkrebs.
- Cukerzis, J.M., 1988. *Astacus astacus* in Europe. Freshwater Crayfish. Biology.
- Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K., Pedersen, P.B., 2013. Farming different species in RAS in Nordic countries: Current status and future perspectives. Aquacultural Engineering. 53, 2-13.
- Edgerton, B.F., Evans, L.H., Stephens, F.J., Overstreet, R.M., 2002. Synopsis of freshwater crayfish diseases and commensal organisms. Aquaculture. 206, 57-135.
- Edsman, L., Füreder, L., Gherardi, F., Souty-Grosset, C., 2010. *Astacus astacus* - Status report, The IUCN Red List of Threatened Species. International Union for Conservation of Nature and Natural Resources.
- Espinal, C.A., Matulić, D., 2019. Recirculating Aquaculture Technologies. 35-76.
- FAO, 2012. Feeding the Growing Aquaculture Sector: An Analysis. in: Aquaculture, S.-C.o. (Ed.). FAO - Committee on Fisheries, Cape Town, South Africa.
- FAO, 2018. The State of World Fisheries and Aquaculture - Meeting the Sustainable Development Goals. Licence: CC BY-NC-SA 3.0 IGO.

- Füreder, L., 2009. Flusskrebse Biologie - Ökologie - Gefährdung. Folio Verlag, Wien.
- Hager, J., 2003. Edelkrebse - Biologie, Zucht, Bewirtschaftung, 2., überarbeitete Auflage ed. Leopold Stocker Verlag, Graz - Stuttgart.
- Holdich, D.M., Reynolds, J.D., Souty-Grosset, C., Sibley, P.J., 2009. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowl. Managt. Aquatic Ecosyst.*, 11.
- Holdich, D.M., Reynolds, J.D., Souty-Grosset, C., Sibley, P.J., 2010. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems*, 11.
- Hutchinson, W., Mathew, J., O'Sullivan, D.D., Casement, D., Clarke, S., 2004. *Recirculating Aquaculture Systems: Minimum Standards for Design, Construction and Management*. Inland Aquaculture Association of South Australia Inc., South Australia, Kent Town.
- IUCN, 2019. *The IUCN Red List of Threatened Species*.
- Jussila, J., Makkonen, J., Vainikka, A., Kortet, R., Kokko, H., 2011. Latent crayfish plague (*Aphanomyces astaci*) infection in a robust wild noble crayfish (*Astacus astacus*) population. *Aquaculture*. 321, 17-20.
- Keller, M., 2017. Die Rolle der Krebse in früheren Jahrhunderten, forum flusskrebse - Historisches zum Flusskrebs. Forum Flusskrebse, Bad Münstereifel.
- Kozák, P., Füreder, L., Kouba, A., Reynolds, J., Souty-Grosset, C., 2011. Current conservation strategies for European crayfish. *Knowledge and Management of Aquatic Ecosystems*, 01.
- Kozák, P., Duris, Z., Petrusek, A., Buric, M., Horka, I., Kouba, A., Kozubikova-Balcarova, E., Policar, T., 2015. *Crayfish Biology and Culture*. University of South Bohemia, Vodnany.
- LAVES, 2011. Vollzugshinweise zum Schutz von Krebsarten in Niedersachsen: Edelkrebs (*Astacus astacus*) - Niedersächsische Strategie zum Arten- und Biotopschutz. in: *Binnenfischerei*, D. (Ed.). Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit – LAVES, Hannover, pp. 12.
- Lennard, W., Goddek, S., 2019. *Aquaponics: The Basics*. 113-143.
- Maiwald, T., 2007. Verdrängung autochthoner Flusskrebarten durch invasive gebietsfremde Flusskrebarten in Mitteleuropa. in: Korn, H., Feit, U. (Eds.), *Treffpunkt Biologische Vielfalt VII - Interdisziplinärer Forschungsaustausch im Rahmen des Übereinkommens über die biologische Vielfalt*. BfN, Bonn, pp. 119-126.

- Möllers, F., 2013. Zwischenbericht Artenschutzprojekt „Edelkrebse in der Nette“ der NWA e.V. Niedersächsisch Westfälische Anglervereinigung e.V., Wallenhorst-Rulle.
- Murray, F., Bostock, J., Fletcher, M., 2014. Review of RAS technologies and their commercial application. Insitute of Aquaculture, Stirling.
- Murray, F., Bostock, J., Fletcher, M., 2018. An update on the 2014 report: “Review of Recirculation Aquaculture System Technologies and their Commercial Application”. Insitute of Aquaculture, Glasgow.
- Oidtmann, B., Cerenius, L., Schmid, I., Hoffmann, R., Söderhäll, K., 1999. Crayfish plague epizootics in Germany - classification of two German isolates of the crayfish plague fungus *Aphanomyces astaci* by random amplification of polymorphic DNA. Diseases of Aquatic Organisms. 35, 235-238.
- OIE, 2019. Infection with *Aphanomyces astaci* (Crayfish plague), Manual of Diagnostic Tests for Aquatic Animals. The World Organisation for Animal Health (OIE), Paris, pp. 18.
- Palm, H.W., Knaus, U., Appelbaum, S., Strauch, S.M., Kotzen, B., 2019. Coupled Aquaponics Systems. 163-199.
- Peay, S., Füreder, L., 2011. Two indigenous European crayfish under threat – how can we retain them in aquatic ecosystems for the future? Knowledge and Management of Aquatic Ecosystems, 33.
- Peer, M., Pfeiffer, M., 2008. Erfassung der Bestandssituation der Flusskrebse im Freistaat Sachsen - Möglichkeiten für Schutz- und Entwicklungsmaßnahmen. Sächsisches Landesamt für Umwelt, Landwirtschaft und Geologie, Dresden.
- Phillips, M., 2018. Mariculture Overview.
- Reynolds, J., Souty-Grosset, C., 2011. Management of Freshwater Biodiversity: Crayfish as Bioindicators. Cambridge University Press.
- Ridler, N., Wowchuk, M., Robinson, B., Barrington, K., Chopin, T., Robinson, S., Page, F., Reid, G., Szemerda, M., Sewuster, J., Boyne-Travis, S., 2007. Integrated Multi – trophic Aquaculture (Imta): A Potential Strategic Choice for Farmers. Aquaculture Economics & Management. 11, 99-110.
- Sander, M., 1998. Aquarientechnik im Süß- und Seewasser. Ulmer, Stuttgart.
- Schrimpf, A., Schulz, H.K., Theissing, K., Pârvolescu, L., Schulz, R., 2011. The first large-scale genetic analysis of the vulnerable noble crayfish *Astacus astacus* reveals low haplotype diversity in central European populations. Knowledge and Management of Aquatic Ecosystems, 35.

- Schrimpf, A., Pârvulescu, L., Copilas-Ciocianu, D., Petrussek, A., Schulz, R., 2012. Crayfish plague pathogen detected in the Danube Delta- a potential threat to freshwater biodiversity in southeastern Europe. *Aquatic Invasions*. 7, 503-510.
- Taylor, H.M., Kluger, L.C., 2016. Aqua- and mariculture management – a holistic perspective on best practices. in: Salomon, T.M.a.M. (Ed.), *Marine Environment Protection*. Springer, Heidelberg.
- Telesetsky, A., 2017. Overfishing and Bycatch, *International Farm Animal, Wildlife and Food Safety Law*, pp. 405-431.
- Vaeßen, S., Groß, H., Nowak, M., 2016. Konzepte zum Schutz des Edelkrebse vor dem Signalkrebs, *forum flusskrebse*. Forum Flusskrebse, Bad Münstereifel.
- Viljamaa-Dirks, S., Heinikainen, S., Nieminen, M., Vennerstrom, P., Pelkonen, S., 2011. Persistent infection by crayfish plague *Aphanomyces astaci* in a noble crayfish population. *Bulletin of the European Association of Fish Pathologists - A case report*. 31, 182-188.
- Walther, M., 2008. *Krebstiere (Crustacea) – Biologie, Vorkommen, Haltung und Erkrankungen, sowie ihre Bedeutung als Zootierobjekte und Lebensmittelressourcen. Eine Literaturstudie.*, Fachbereich Veterinärmedizin. Justus-Liebig-Universität Gießen, Gießen, pp. 381.
- Wik, T., Lindén, B., 2004. *Modeling, Control and Simulation of Recirculating Aquaculture Systems*. IFAC Proceedings Volumes. 37, 141-146.

2. Research Background Biology of candidate Noble crayfish *Astacus astacus*

Taxonomy: Arthropoda/Crustacea/Malacostraca/Decapoda/Astacidea/Astacus:
A. astacus

The Noble crayfish *Astacus astacus* is one of six native crayfish in Europe (ICS) and was once found in nearly all freshwater systems like streams, rivers and standing waters (Ingle, 1997; Westman, Savolainen, 2001). It is the biggest native crayfish with up to 350 g, 15 cm in length without claw, can reach an age of 20 years and was previously a common food source in Europe (Cukerzis, 1988; Holdich, 2002; Skurdal, Taugbøl, 2001). Animals are active at twilight and night and hide in shelters over the day and winter period when feed intake is reduced (Blohm, Gaumert, Kämmerleit, 1994; Pöckl, 1998a). With raising water temperature in spring crayfish activity increases until autumn when breeding takes place (Westin, Gydemo, 1986). As an omnivore feeder the Noble crayfish is known as a natural habitat manager and converter of detritus and ailing fish (Pöckl, 1998a; Wittmaack, 2006). While this crayfish is an indicator for healthy and stable waters, non-natural water constructions, pollution and fertilization are drivers to repress populations (Reynolds, Souty-Grosset, 2011; Skurdal, Taugbøll, 1994). Especially fertilization in agriculture areas leads to a decrease (Reynolds, Souty-Grosset, 2011).

A natural proliferation does hardly take place since these crayfish remain in their habitat (Zimmermann, 2012). Thus, especially quick environmental changes can lead to drastic slump in population numbers. In the late 19th century several alien crayfish species were introduced in Europe and with them a far more deadly threat, the highly infectious crayfish plague (*Aphanomyces astaci*) (LAVES, 2011). Until now, most native crayfish populations vanished and a once common food source with over 5 million crayfish eaten per year (1853 - 1879) e.g. in Paris has become a vulnerable species on the IUCN Red List (Basen,

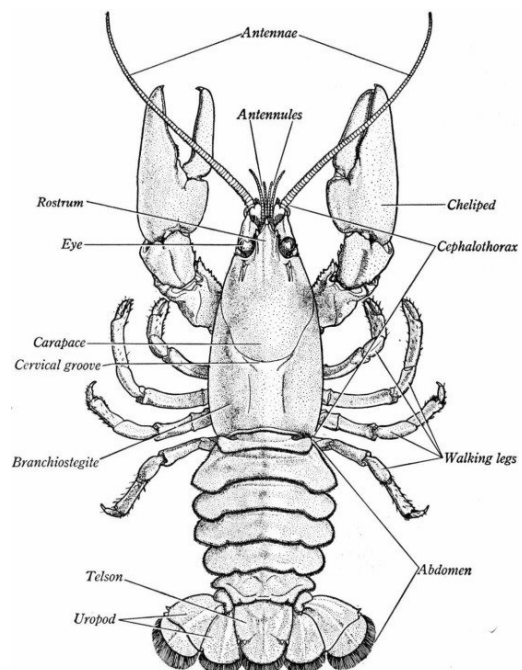


Figure 3 Crayfish anatomy and body structure.
Source: <http://www.jouefct.com>

Chucholl, 2015; Edgerton, Evans, Stephens, Overstreet, 2002; Holdich, 2002; Holdich, Reynolds, Souty-Grosset, Sibley, 2010; Westman, Savolainen, 2001). Other factors like water

pollution and habitat alteration and destruction have subsidized this progress (Edsman, Füreder, Gherardi, Souty-Grosset, 2010; Gherardi, 2011). Today only few wild populations are existent and can be found in secluded areas (Füreder, 2009). In addition, small farms are operated where crayfish are produced for restocking purposes in limited numbers and without a focus on preserving genetic populations and their local affiliation.

Morphology

The crayfish is covered with an exoskeleton consisting mostly of chitin and calcium carbonate while the former can be separated into four plates. These plates are connected via flexing membranes and allow movement of the crayfish. The body itself can be separated into two parts, the cephalothorax and the abdomen (s. Fig. 3). The cephalothorax covered by the carapace as a shield and contains the internal organs and includes post orbital ridges and the cervical groove as crayfish typical features. Antenna and eyes as part of the rostrum belong to the cephalothorax as well. As part of the decapods Noble crayfish has ten five pairs of pereopods including the chelae which arise from one body segment each. The abdominal segments as well as the appendages are connected by joints (Keller, 2017; Pöckl, 1998a). This allows the exact positioning of pereopods within its hard shell. Chelae are used to grab food and transferred to the mouth by the pereopods (Kozák, Duris, Petrussek, Buric, Horka, Kouba, Kozubikova-Balcarova, Policar, 2015). The Chelae also are used for various other actions like signaling, defending, attacking, climbing, constructing and digging. The surface of the carapace of each crayfish species is different and allows precise identification with other characteristics of e.g. chela color and surface (Fischereibehörde, 2012).

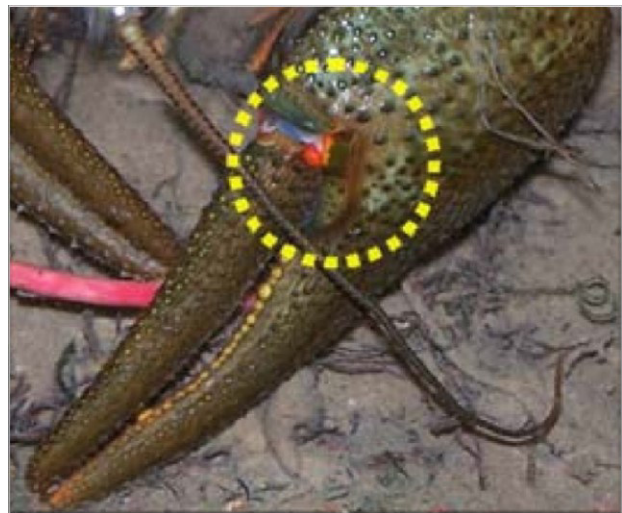


Figure 4 Chela of *A. astacus* with characteristic notch and red cutaneous joint (yellow circle). Source: H. H. Arzbach.

For Noble crayfish a granular surface, red ventral dots on the chelae joints and a notch in the chelae finger are characteristically (s. Fig. 4).

Water parameters and (artificial) feed

Noble crayfish is an omnivorous feeder but in general a vegetable diet prevails (Haase, Heidecke, Klapperstück, 1989). Preferred are algae and soft-leaved water plants like

Stoneworts (Charophyta), Water Moss (Fontinalaceae) or Water Starwort (Callitriche). Remarkably, crayfish can be quite delicate as handier food is preferred against nutrient content as well as decomposing material against fresh one (D'Agaro, Renai, Gherardi, 2004). Larvae of insects (e.g. trichoptera larva), water snails and leech are used as animal source foods (Ackefors, 2000; Axelsson, Nyström, Sidenmark, Brönmark, 1997; Pöckl, 1998b). Even if healthy fish is not accessible as a food source removal of ill or dead fish by crayfish is an important factor in the ecosystem (Hager, 2003; Reynolds, Souty-Grosset, 2011). Cannibalism is less important at least in natural environment while it plays a crucial role in OPS and RAS layouts and economics. An oxygen level of >60 % is recommended (Hager, 2003; Jeske, 2010). *A. astacus* is relatively tolerant in water parameters and can survive in water bodies of moderate water quality, commonly described as quality grade II¹, while quality grade I in most cases is too cold and/or low in nutrients (Arle, Blondzik, Claussen, Duffek, Grimm, Hilliges, Kirschbaum, Kirst, Koch, Koschorreck, Lepom, Leujak, Mohaupt, Naumann, Pirntke, Rechenberg, Schilling, Ullrich, Wellnitz, Werner, Wolte, 2017; Haase, Heidecke, Klapperstück, 1989; Peer, Pfeiffer, 2008). Noble crayfish prefer a temperature between 18-21 °C, a maximum of 25 °C but needs at least 15 °C in summer over a longer period for genitals development while lower degrees are needed for egg evaluation in winter (Arzbach, 2010; Cukerzis, 1973; Hager, 2003). A pH between 5-10 (Cukerzis, 1988; Jeske, 2010), a total hardness between 7-9 and carbonate hardness between of 11 are recommendable (Jeske, 2010). Low pH values, especially under 5, lead to problems in shell structure (Hager, 2003). The optimum of ammonium (NH⁴⁺) is under 2 mg/l (Avery, Romaine, McClain, 1998; Wickins, 2002), for nitrite under 1.6 (McClain, 2012; Wickins, 2002) for crayfish in general and nitrate should be under 20 mg/l (Jeske, 2010). Noble crayfish can accept higher and lower values even over longer periods. Towards pollution and environmental toxins e.g. via agricultural fertilization and insecticides *A. astacus* reacts quite sensible on fertilization even if measured in low quantities which caused many populations to perish after the upcoming up modern and excessive agriculture farming (Reynolds, Souty-Grosset, 2011). Reported feed requirements for freshwater crayfish in general include a lipid content of about 10 %, and a protein content

¹ Güteklasse II describes water bodies of moderate pollution with e.g. good oxygen supply (> 6 mg/l) and a high species diversity. This German classification considers water quality, (over-)fertilization and the Saprobien-Index which represents characteristic organisms Arle, J., Blondzik, K., Claussen, U., Duffek, A., Grimm, S., Hilliges, F., Kirschbaum, B., Kirst, I., Koch, D., Koschorreck, K., Lepom, P., Leujak, W., Mohaupt, V., Naumann, S., Pirntke, U., Rechenberg, J., Schilling, P., Ullrich, A., Wellnitz, J., Werner, S., Wolte, R., 2017. Gewässer in Deutschland: Zustand und Bewertung. Umweltbundesamt, Dessau-Roßlau, Wagner, R., 2006. Wasserqualität und Gewässerqualität. Limnologische Fluss-Station Schlitz..

of between 25 – 30 % (Jover, Fernandez-Carmona, Del Rio, Soler, 1999; Valipour, Ozorio, Shariatmadari, Abedian, Seyfabadi, Zahmatkesh, 2012; Xu, Liu, Shen, Li, Wang, Zhang, 2013) and these are also suggested for *A. astacus* (Ackefors, Castell, Boston, Rätty, Svensson, 1992). Ackefors (1992) also measured fatty acid composition and content of Noble crayfish and stated the capability to synthesize fatty acids on their own. Although there is no reported specification for *A. astacus* and no commercial feed available for the species the basic nutritional requirements outlined above are supplied by various industrial carp feeds.

Growth, activity and shelter

Noble crayfish do not grow constantly due to their hard shell but moult lifelong in regular intervals depending on age, feed and water temperature. After hatch young crayfish discard their shell approximately seven till nine times which decreases to one or two adult moults per year (Hager, 2003; Taugbøl, Skurdal, 1992). Moulting is regulated by hormones and can be induced by light regime (Westin, Gydemo, 1986). Evolutionary induced these moults usually take place over the day when most crayfish rest (Franke, Wessels, Horstgen-Schwark, 2011; Westin, Gydemo, 1986). Franke, Hoerstgen-Schwark (2013) stated moon cycle and light dependent moults while these can also be induced by constant light exposure and stress (Franke, Wessels, Horstgen-Schwark, 2011; Jähne, 2014; Taugbøl, Skurdal, 1988; Westin, Gydemo, 1986). The knowledge about simultaneous moults would be helpful for crayfish culture if applicable to the system since most losses are referable to crayfish-crayfish interactions. This accounts for OPS as well as for RAS culture. As Noble crayfish is a night and twilight active species it needs kinds of shelter where they can rest over the day and which are easy to defend. A shelter is also mandatory for and especially after molting when crayfish are extremely vulnerable towards predatory fish and conspecifics. *A. astacus* uses natural coverage under roots and stones for this purpose but if not available they are able to build and dig them themselves. Thus, Noble crayfish is bound to the littoral zone and population density is given by the abundance of usable shelter structures and soil. A natural water body with a high variety in depth and width profile with steep littoral parts and hard but dig through substrates (e.g. clay and hardpan) provides excellent shelter conditions (Hager, 2003). Soft and muddy bottoms or bottoms subject to constant rearrangements are disadvantageous (Haase, Heidecke, Klapperstück, 1989). Noble crayfish used to have at least two or three individual shelters in their territory (Blohm, Gaumert, Kämmerleit, 1994). Especially adults have specific shelters in steep areas which they regularly use and check while younger crayfish

prefer hiding in shallow and or overgrown areas (Blohm, Gaumert, Kämmerleit, 1994; Vorburger, Ribi, 1999). Crucial for an artificial shelter is the diameter which has to fit to the individual crayfish size and offering only one entrance. The entrance is blocked with the most durable part of the animal, the claws. Together with other appendices the crayfish can tuck itself into a good fitting shelter and is almost unmovable. Even in OPS where perforated sand bricks are used it is extremely difficult to get the animals out of their holes (Hager, 2003).

Breeding

Reproduction of Noble crayfish is bound to several factors. The water temperature must be over 15 °C in summer to allow development of sexual organs of male and female crayfish in their 2nd to 3rd year. Time of sexual maturity in nature depends on age but is not dependent on crayfish size. Breeding occurs once per year from autumn to spring depending on the climate and temperature. In general, it starts with pheromone induced mating of the crayfish in October – November. Male crayfish turn over the females and attach a package of spermatophores next to the female vent (Hager, 2003). After some days or even weeks depending on breeding time and climate the ovulated eggs are fertilized by the sperm and glued to the hairs of the abdominal pleopods where a mucus-tent is secreted by the female (Reynolds, Souty-Grosset, 2011). The mucus also activates the sperm by softening the sperm packages and releasing the sperms. Around 50-200 eggs are attached to the pleon after fertilization and cared of by the female crayfish (Hager, 2003) which supply fresh and oxygenic water via the swimmerets and remove dead or fungal infected eggs. A temperature of under 4°C is needed over a period of 10-14 days to induce further egg development. Egg development takes approximately 1.550 CTU (Celsius Temperature Units = degrees Celsius * days) (Policar, Simon, Kozál, 2004) . These factors lead to a very climate depending hatch which takes place between April-July. The young crayfish hatch fully developed with few millimeters in length without free larval stages (Cukerzis, 1973). Females reduce feed intake drastically, stay in their shelters and do not moult until hatch (Taugbøl, Skurdal, 1990).

2.2. Protective measures and economic value

Various projects have been conducted so far to prohibit the spread of NICS in Europe to protect ICS in general. Most of them included technical issues to prevent the progress of NICS in rivers with e.g. traps and stream barriers (Chucholl, 2012; Krieg, 2013; Krieg, Zenker, 2014; Vaeßen, Groß, Nowak, 2016). Since crayfish are good clamberer these barriers need to be well

planned for each building measure which is a cost intensive approach especially when considering the spread of NICS in Europe and the amount of barriers needed (Chucholl, DümpeImann, 2017; Vaeßen, Groß, Nowak, 2016). A workgroup of the University of Koblenz-Landau aims to identify the genetic variety of crayfish species. Results identify several populations of ICS with partly different genetic profiles and also different *Astacus astacus* strains (Schrimpf, Theissing, Dahlem, Maguire, Pârvulescu, Schulz, Schulz, 2014; Schrimpf, Piscione, Cammaerts, Herman, Collas, Jung, Ottburg, Roessink, Rollin, Schulz, Theissing, 2017). These subspecies and the preservation of their genetic pool is seen mandatory for the survival of endangered crayfish especially in regard to climate change (Groß, 2015; Kerth, Fischer, Fleischer, Limberg, Blüthgen, Dworchak, Dittrich, Rödel, Obermaier, 2015). In Switzerland, a federal office for crayfish coordination (KFKS) has been established at the University of Life Science to coordinate crayfish projects according to the national action schedule (Stucki P., B., 2011).

In spite of the danger of the crayfish plague, Noble crayfish is still of commercial interest due to its high meat content and quality. Limited availability has increased the economic value of this once common consumer good to a luxury food product available in small quantities only on from the remaining farms for 35-50 €/kg live weight (Ebeling, 2014; Franke, Wessels, Horstgen-Schwark, 2011; Kühn, 2015; Skurdal, Taugbøl, 2001). For German farmers the Scandinavian market and the *Kräftskiva*² offer up to 70 €/kg. Since all farms run a high risk of plague infection and are therefore pretty small, the market for juvenile crayfish for restocking purposes offer more secure and better yields of 0,7-2,5 €/animal. As a result the overall amount of crayfish as a food product is several 100 kg per year while +20.000 summerlings are sold as stocking material (Kühn, 2015).

2.3. Cultivation and techniques

The first crayfish farm described is a crayfish enclosure, which can be seen as a forerunner of an OPS (Keller, 2017). The actual method for culturing *A. astacus* and other crayfish species remains OPS (Ackefors, 2000; Cukerzis, 1988; Huner, 1994; O'Sullivan, Jones, Fielder, 2012; Wickins, 2002), where sufficient space is available to reduce cannibalism and food is, at a minimum, partially supplied by the pond itself. These systems are exposed to local

² Kräftskiva is a traditional Swedish celebration day at the end of summer marking as well the end of the crustacean catch season where lots of crayfish are eaten.

environmental conditions (i.e. temperature, feed), the economic risk of a crayfish plague infection is high and the growth period is limited to a maximum of six months per year with extremely low production levels (Füreder, 2009; Skurdal, Taugbøll, 1994). In addition, the intensive work load and the mortality rates of up to 90 % due to predation and cannibalism are limiting factors for large farms (Daws, Grills, Konzen, Moore, 2002; Dethlefs, 2007; Hager, 2003; Usio, Konishi, Nakano, 2001). Bricks with different perforation sizes are used for shelter substitutes (Hager, 2003). In general, one technique is used in all farms to enhance breeding success in OPS: males and at least two females are placed in separate tanks in autumn. After mating male crayfish are removed from the breeding tank and females care for the eggs until hatching. In spring, these tanks warm up faster than the bigger ponds and egg development proceeds faster. Females are then removed as well while young crayfish profit from higher water temperature as well which results in a faster growth within their first months. Often run in a bypass within the OPS, survival rates are still pretty low at approximately 10 % due to insects like great diving beetle or mosquito larvae. A separate water cycle and re-movement of predatory insects enhance survival rates significantly (Göckemeyer, 2014).

Recirculating aquaculture system and their potential

So far various scientific research was conducted to enhance the efficiency of *A. astacus* culture techniques in recirculating aquaculture systems (RAS). High stocking densities with 14-month-old juveniles of up to 1.560 individuals per m² were tested in RAS. Controlled light and temperature regime and artificial feed led ensured a survival rate of more than 90% (Franke, Wessels, Horstgen-Schwark, 2011). Continuing artificial summer conditions were tested to suppress the hibernal resting period between October and April and led to a delay of this period with further moults (Franke, Hoerstgen-Schwark, 2013). Franke, Hoerstgen-Schwark (2013) also presented first findings that the lunar photic stimuli do indirectly act as a trigger and could entrain an endogenous molting rhythm to the lunar cycle. The influence of social factors on the nocturnal activity pattern was observed and showed the repression of smaller crayfish by bigger one which could lead to a large variation in individual growth rates of equal aged animals. It is suggested that grading could enhance the overall growth (Franke, Hörstgen-Schwark, 2015).

No specialized RAS adapted to the needs of the *A. astacus* are available despite other crustacean species, such as the European lobster (*Homarus gammarus*) and the Australian crayfish (*Cherax quadricarinatus*), being partly or fully cultured in RAS (Barki, Karplus, Manor,

Parnes, Aflalo, Sagi, 2006; Knudsen, Tveite, 1999; Manor, Segev, Leibovitz, Aflalo, Sagi, 2002; Perez Benavente, Uglem, Browne, Marino Balsa, 2010). This is due to unknown culture conditions, natural behavior, workload and operating costs. *Orconectes limosus* is already cultured in RAS (Auvergne, 1979) but is much smaller and barely cannibalistic in contrast to *Astacus astacus* (Kozák, Buřič, Policar, Hamáčková, Lepičová, 2007).

As *A. astacus* has not been economically produced in RAS to date, such a system must be developed in accordance with species needs and given restrictions for animal welfare and food production in the European Union. Beside the RAS design and e.g. shelter solutions the nutritional requirements of crayfish have to be analyzed and whether these can be covered with existing industrial fish feeds. A further aspect is the cannibalistic behavior at sexual maturity after the third year. This must be prevented to realize higher stockings densities in comparison to OPS which allow densities of at least two animals per m² (Hager, 2003). After the development of a suitable RAS this aspect is crucial to provide a complete production cycle in cost-intensive RAS.

References

- Ackefors, H., Castell, J.D., Boston, L.D., Rätty, P., Svensson, M., 1992. Standard experimental diets for crustacean nutrition research. II. Growth and survival of juvenile crayfish *Astacus astacus* (Linné) fed diets containing various amounts of protein, carbohydrate and lipid. *Aquaculture*. 104, 341-356.
- Ackefors, H.E.G., 2000. Freshwater crayfish farming technology in the 1990s: a European and global perspective. *Fish and Fisheries*. 1, 337-359.
- Arle, J., Blondzik, K., Claussen, U., Duffek, A., Grimm, S., Hilliges, F., Kirschbaum, B., Kirst, I., Koch, D., Koschorreck, K., Lepom, P., Leujak, W., Mohaupt, V., Naumann, S., Pirntke, U., Rechenberg, J., Schilling, P., Ullrich, A., Wellmitz, J., Werner, S., Wolte, R., 2017. *Gewässer in Deutschland: Zustand und Bewertung*. Umweltbundesamt, Dessau-Roßlau.
- Arzbach, H.-H., 2010. *Biologie, Ökologie und Verhalten der Flusskrebse*. Krebsseminar Echem, Echem.
- Auvergne, A., 1979. Culture of crayfish, L'elevage des ecrevisses. Eds. du Point Veterinaire, Maisons-Alfort (France), pp. 87.
- Avery, J.L., Romaine, R.P., McClain, W.R., 1998. *Crawfish Production Production Economics, Pond Construction and Water Supply*. Southern Regional Aquaculture Center, 4.
- Axelsson, E., Nyström, P., Sidenmark, J., Brönmark, C., 1997. Crayfish predation on amphibian eggs and larvae. *Amphibia-Reptilia*. 18, 217-228.
- Barki, A., Karplus, I., Manor, R., Parnes, S., Aflalo, E.D., Sagi, A., 2006. Growth of redclaw crayfish (*Cherax quadricarinatus*) in a three-dimensional compartments system: Does a neighbor matter? *Aquaculture*. 252, 348-355.
- Basen, T., Chucholl, C., 2015. *Harte Schale, leckerer Kern - Krebse und Muscheln als Lebensmittel*.
- Blohm, H.-P., Gaumert, D., Kämmerleit, M., 1994. Leitfaden für die Wieder- und Neuansiedlung von Fischarten. *Binnenfischerei in Niedersachsen*. Niedersächsisches Landesamt für Ökologie, Hildesheim, pp. 90.
- Chucholl, C., 2012. Notorisch gefährdet: Probleme, Strategien und Perspektiven im Artenschutz für Edelkrebse, SVK-Fischereitagung, Fulda, pp. 18.
- Chucholl, C., Dümpelmann, C., 2017. *Sondergutachten 2017: Erstellung einer Expertise zu Krebsperren und alternativen Schutzmaßnahmen für den Steinkrebs*.
- Cukerzis, J.M., 1973. Biologische Grundlagen der Methode der künstlichen Aufzucht der Brut des *Astacus astacus* L. *Freshwater Crayfish*. 1, 187-201.

- Cukerzis, J.M., 1988. *Astacus astacus* in Europe. Freshwater Crayfish. Biology.
- D'Agaro, E., Renai, B., Gherardi, F., 2004. Evaluation of the American waterweed (*Elodea Canadensis* Michx.) as supplemental food for the Noble crayfish, *Astacus astacus*. Bulletin Français de la Pêche et de la Pisciculture, 439-445.
- Daws, A.G., Grills, J., Konzen, K., Moore, P.A., 2002. Previous Experiences Alter the Outcome of Aggressive Interactions Between Males in the Crayfish, *Procambarus Clarkii*. Marine and Freshwater Behaviour and Physiology. 35, 139-148.
- Dethlefs, A., 2007. Predator - Prey - Interaction between Dragonfly Larva (Odonata: *Anisoptera*) and Crayfish Juveniles (*Astacus astacus*), Zoological Institute - Department Limnology. Christian - Albrechts - University Kiel, Kiel, Germany, pp. 124.
- Ebeling, M., 2014. Market prices for *Astacus astacus*. in: Seemann, U.B. (Ed.).
- Edgerton, B.F., Evans, L.H., Stephens, F.J., Overstreet, R.M., 2002. Synopsis of freshwater crayfish diseases and commensal organisms. Aquaculture. 206, 57-135.
- Edsman, L., Füreder, L., Gherardi, F., Souty-Grosset, C., 2010. *Astacus astacus* - Status report, The IUCN Red List of Threatened Species. International Union for Conservation of Nature and Natural Resources.
- Fischereibehörde, S.L.f.L.-. 2012. Flusskrebse - Bestimmungsschlüssel.
- Franke, R., Hoerstgen-Schwark, G., 2013. Lunar-rhythmic molting in laboratory populations of the Noble Crayfish *Astacus astacus* (Crustacea, Astacidea): an experimental analysis. PLoS One. 8, e68653.
- Franke, R., Hörstgen-Schwark, G., 2015. Influence of social factors on the nocturnal activity pattern of the noble crayfish, *Astacus astacus* (Crustacea, Decapoda) in recirculating aquaculture systems. Aquaculture Research. 46, 2929-2937.
- Franke, R., Wessels, S., Horstgen-Schwark, G., 2011. Enhancement of survival and growth in crowded groups: the road towards an intensive production of the noble crayfish *Astacus astacus* L. in indoor recirculation systems. Aquaculture Research. 44, 451-461.
- Füreder, L., 2009. Flusskrebse Biologie - Ökologie - Gefährdung. Folio Verlag, Wien.
- Gherardi, F., 2011. Towards a sustainable human use of freshwater crayfish (Crustacea, Decapoda, Astacidea). Knowl. Managt. Aquatic Ecosyst., 02.
- Göckemeyer, S., 2014. How to reduce extensive loss of juvenily crayfish in OPS. in: Seemann, U.B. (Ed.), Poggenhagen.

- Groß, H., 2015. Was bringen genetische Untersuchungen von Flusskrebse für den Artenschutz?, forum flusskrebse. Forum Flusskrebse, Bad Münstereifel.
- Haase, T., Heidecke, D., Klapperstück, J., 1989. Zur Ökologie und Verbreitung des Edelkrebse *Astacus astacus* in der DDR Hercynia N. F. 26, 21.
- Hager, J., 2003. Edelkrebse - Biologie, Zucht, Bewirtschaftung, 2., überarbeitet Auflage ed. Leopold Stocker Verlag, Graz - Stuttgart.
- Holdich, D.M., 2002. Distribution of crayfish in Europe and some adjoining countries. Bulletin Français de la Pêche et de la Pisciculture, 611-650.
- Holdich, D.M., Reynolds, J.D., Souty-Grosset, C., Sibley, P.J., 2010. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. Knowledge and Management of Aquatic Ecosystems, 11.
- Huner, J.V., 1994. Cultivation of freshwater crayfishes in North America. in: Huner, J.V. (Ed.), Freshwater Crayfish Aquaculture in North America, Europe, and Australia. The Haworth Press, New York, pp. 5-156.
- Ingle, R., 1997. Crayfishes, lobsters and crabs of Europe - An illustrated guide to common and traded species. Springer.
- Jähne, G., 2014. Kultur des europäischen Edelkrebse (*Astacus Astacus*) in landbasierten Kreislaufsystemen: Größenklassensortierung und technische Anpassungen zur Verbesserung der Kulturbedingungen, Aquakultur und Sea-Ranching. Universität Rostock, Rostock, pp. 153.
- Jeske, H., 2010. Edelkebs *Astacus astacus* - Zucht, Vermehrung und Bewirtschaftung von Edelkrebse. Krebsseminar, Echem.
- Jover, M., Fernandez-Carmona, J., Del Rio, M., Soler, M., 1999. Effect of feeding cooked-extruded diets, containing different levels of protein, lipid and carbohydrate on growth of red swamp crayfish (*Procambarus clarkii*). Aquaculture. 178, 127-137.
- Keller, M., 2017. Die Rolle der Krebse in früheren Jahrhunderten, forum flusskrebse - Historisches zum Flusskrebse. Forum Flusskrebse, Bad Münstereifel.
- Kerth, G., Fischer, K., Fleischer, T., Limberg, J., Blüthgen, N., Dworchak, K., Dittrich, C., Rödel, M., Obermaier, E., 2015. Anpassungskapazität von 50 Arten mit potentiell hohem Austerberisiko gegenüber dem Klimawandel in Deutschland. Natur und Landschaft. 90, 17-24.
- Knudsen, H., Tveite, S., 1999. Survival and growth of juvenile lobster *Homarus gammarus* L. raised for stock enhancement within in situ cages. Aquaculture Research. 30, 421-425.

- Kozák, P., Buřič, M., Policar, T., Hamáčková, J., Lepičová, A., 2007. The effect of inter- and intra-specific competition on survival and growth rate of native juvenile noble crayfish *Astacus astacus* and alien spiny-cheek crayfish *Orconectes limosus*. *Hydrobiologia*. 590, 85-94.
- Kozák, P., Duris, Z., Petrusek, A., Buric, M., Horka, I., Kouba, A., Kozubikova-Balcarova, E., Policar, T., 2015. *Crayfish Biology and Culture*. University of South Bohemia, Vodnany.
- Krieg, R., 2013. *Krebssperren in kleineren Gewässern*, Sissach.
- Krieg, R., Zenker, A., 2014. *Krebssperren: Feldversuche in einer Fischtreppe, einem natürlichen und einem verbauten Gewässer*. Fachhochschule Nordwestschweiz, Muttenz.
- Kühn, C., 2015. *Einbindung einer Edelkrebsaquakultur in ein integriertes Aquakultursystem am Standort Hohen Wangelin in den Jahren 2012 - 2015*. Institut für Fischerei, Rostock.
- LAVES, 2011. *Vollzugshinweise zum Schutz von Krebsarten in Niedersachsen: Edelkrebs (*Astacus astacus*) - Niedersächsische Strategie zum Arten- und Biotopschutz*. in: *Binnenfischerei*, D. (Ed.). Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit – LAVES, Hannover, pp. 12.
- Manor, R., Segev, R., Leibovitz, M.P., Aflalo, E.D., Sagi, A., 2002. Intensification of redclaw crayfish *Cherax quadricarinatus* culture: II. Growout in a separate cell system. *Aquacultural Engineering*. 26, 263-276.
- McClain, W.R., 2012. *Crawfish Production: Pond Construction and Water Requirements*. in: Center, S.R.A. (Ed.), pp. 6.
- O'Sullivan, D.D., Jones, C., Fielder, D., 2012. *Freshwater Crustaceans*. in: Lucas, J.S., Southgate, P.C. (Eds.), *Aquaculture: Farming Aquatic Animals and Plants*. Wiley-Blackwell, pp. 948 (420-442).
- Peer, M., Pfeiffer, M., 2008. *Erfassung der Bestandssituation der Flusskrebse im Freistaat Sachsen - Möglichkeiten für Schutz- und Entwicklungsmaßnahmen*. Sächsisches Landesamt für Umwelt, Landwirtschaft und Geologie, Dresden.
- Perez Benavente, G., Uglem, I., Browne, R., Marino Balsa, C., 2010. Culture of juvenile European lobster (*Homarus gammarus* L.) in submerged cages. *Aquaculture International*. 18, 1177-1189.
- Pöckl, M., 1998a. *Nahrung und Ernährung der Flußkrebse*. *Stapfia* 58. 137.
- Pöckl, M., 1998b. *Funktionelle Morphologie und Anatomie der Flußkrebse*. *Biologiezentrum Linz, Austria, Linz*, pp. 131-142.

- Polcar, T., Simon, V., Kozál, P., 2004. Egg Incubation in the Noble Crayfish (*Astacus astacus* L.): The Effect of Controlled Laboratory and Outdoor Ambient Condition on Hatching Success, Growth and Survival Rate of Juveniles. Bulletin Francais de la Peche et de la Pisciculture.
- Reynolds, J., Souty-Grosset, C., 2011. Management of Freshwater Biodiversity: Crayfish as Bioindicators. Cambridge University Press.
- Schrimpf, A., Theissinger, K., Dahlem, J., Maguire, I., Pârvulescu, L., Schulz, H.K., Schulz, R., 2014. Phylogeography of noble crayfish (*Astacus astacus*) reveals multiple refugia. Freshwater Biology. 59, 761-776.
- Schrimpf, A., Piscione, M., Cammaerts, R., Herman, D., Collas, M., Jung, A., Ottburg, F., Roessink, I., Rollin, X., Schulz, R., Theissinger, K., 2017. Genetic characterization of western European noble crayfish populations (*Astacus astacus*) for conservation management. Conservation Genetics. 18, 1299-1315.
- Skurdal, J., Taugbøll, T., 1994. Biology, Culture and Management of the Noble Crayfish *Astacus Astacus* L.
- Skurdal, J., Taugbøl, T., 2001. Crayfish of commercial importance. in: Holdich, D.M. (Ed.), Biology of Freshwater Crayfish. Blackwell Science, Oxford, pp. 467-510.
- Stucki P., B., Z., 2011. Aktionsplan Flusskrebse Schweiz. Artenförderung von Edelkrebse, Dohlenkrebse und Steinkrebse. Bundesamt für Umwelt, Bern.
- Taugbøl, T., Skurdal, J., 1988. Increased proportion of mature females of the Noble Crayfish (*Astacus astacus* L.) in culture conditions. Aquaculture. 69, 39-42.
- Taugbøl, T., Skurdal, J., 1990. Reproduction, Molting and Mortality of Female Noble Crayfish, *Astacus astacus* (L., 1758), From Five Norwegian Populations Subjected To Indoor Culture Conditions (Decapoda, Astacoidea). Crustaceana. 58, 113-123.
- Taugbøl, T., Skurdal, J., 1992. Growth, mortality and moulting rate of noble crayfish *Astacus astacus* L., juveniles in aquaculture experiments. Aquaculture and Fisheries Management. 23, 411-420.
- Usio, N., Konishi, M., Nakano, S., 2001. Species Displacement Between an Introduced and A 'vulnerable' Crayfish: The Role of Aggressive Interactions and Shelter Competition. Biological Invasions. 3, 179-185.
- Vaeßen, S., Groß, H., Nowak, M., 2016. Konzepte zum Schutz des Edelkrebse vor dem Signalkrebse, forum flusskrebse. Forum Flusskrebse, Bad Münstereifel.

- Valipour, A., Ozorio, R.O.A., Shariatmadari, F., Abedian, A., Seyfabadi, J., Zahmatkesh, A., 2012. Effects of dietary lipid levels on growth, survival, and molting of yearling narrow clawed crayfish, *Astacus leptodactylus*. *Journal of Applied Aquaculture*. 24, 316-325.
- Vorburger, C., Ribí, G., 1999. Aggression and competition for shelter between a native and an introduced crayfish in Europe. *Freshwater Biology*. 42, 111-119.
- Wagner, R., 2006. Wasserqualität und Gewässerqualität. *Limnologische Fluss-Station Schlitz*.
- Westin, L., Gydemo, R., 1986. Influence of light and temperature on reproduction and moulting frequency of the crayfish, *Astacus astacus* L. *Aquaculture*. 52, 43-50.
- Westman, K., Savolainen, R., 2001. Long term study of competition between two co-occurring crayfish species, the native *Astacus astacus* L. and the introduced *Pacifastacus leniusculus* Dana, in a finnish lake. *Bulletin Français de la Pêche et de la Pisciculture*, 613-627.
- Wickins, J.F.L., Daniel O'C. , 2002. *Crustacean Farming: Ranching and Culture*, 2nd Edition ed. Wiley-Blackwell.
- Wittmaack, K.E., 2006. Analyses of nutritional ecology of the Nobel crayfish (*Astacus astacus*) - Diet selection and prey experiments, Zoological Institute - Department Limnology. Christian - Albrechts - University Kiel, Kiel, Germany, pp. 89.
- Xu, W.N., Liu, W.B., Shen, M.F., Li, G.F., Wang, Y., Zhang, W.W., 2013. Effect of different dietary protein and lipid levels on growth performance, body composition of juvenile red swamp crayfish (*Procambarus clarkii*). *Aquaculture International*. 21, 687-697.
- Zimmermann, J.K.M., 2012. Noble Crayfish (*Astacus astacus*) in a Chainging World – Implications for Management, Department of Natural Sciences, Engineering and Mathematics. Mid Sweden University, SE - 851 70 Sundsvall, Sundsvall. Sweden, pp. 39.

Chapter I

Growth performance of Noble crayfish *Astacus astacus* in recirculating aquaculture systems

Seemann UB, Lorkowski K, Slater MJ, Buchholz F, Buck BH



Uli B. Seemann

published in Aquaculture International (2014)

DOI 10.2983/035.036.0223

Due to copyright policies of the publisher the article is linked via the above mentioned DOI.

Chapter II

Feed alternatives for Noble crayfish *Astacus astacus* based on fatty acid and lipid analyses

Seemann UB, Lorkowski K, Slater MJ, Buchholz F, Buck BH



published in Journal of Shellfish Research (2017)

DOI 10.2983/035.036.0223

Feed Alternatives for Noble Crayfish *Astacus astacus* Based on Fatty Acid and Lipid Analyses

Authors: Uli B. Seemann, Kai Lorkowski, Matthew J. Slater, Friedrich Buchholz, and Bela H. Buck

Source: Journal of Shellfish Research, 36(2) : 519-527

Published By: National Shellfisheries Association

URL: <https://doi.org/10.2983/035.036.0223>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

FEED ALTERNATIVES FOR NOBLE CRAYFISH *ASTACUS ASTACUS* BASED ON FATTY ACID AND LIPID ANALYSES

ULI B. SEEMANN,^{1*} KAI LORKOWSKI,^{1,2} MATTHEW J. SLATER,¹ FRIEDRICH BUCHHOLZ¹
AND BELA H. BUCK^{1,2}

¹Aquaculture Research Group, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany; ²Applied Marine Biology, University of Applied Sciences Bremerhaven, An der Karlstadt 8, D-27568 Bremerhaven, Germany

ABSTRACT Commercially viable aquaculture of noble crayfish *Astacus astacus* (Linnaeus, 1758), a once plentiful food species in Europe, requires production of suitable artificial diets for optimal growth in recirculating aquaculture systems (RAS). This technique provides the opportunity to culture this species in an enclosed ecosystem which minimizes the risk of infection—resulting in high-value food products as well as high-quality juveniles for restocking purposes. In the current study, noble crayfish were fed in RAS with a commercial diet and relied on natural forage in an open pond system (OPS) for two months. Energy, lipid, and fatty acid (FA) content of available and alternative diets [Cyprinid K2 (CK2), *Mytilus edulis* Rafinesque, 1815, *Elodea* spp. Michx.) and crayfish tissue were determined. Crayfish from the pond system had significantly ($P < 0.01$ OPS versus all RAS treatments; Pairwise Wilcoxon) higher lipid content (8.51%) and more diverse FA composition than RAS crayfish. At least 15 partly combined FAs were present in crayfish whereas seven were provided by the diet CK2 and 10 by *M. edulis* and *Elodea* spp. In addition to the recommended lipid (<10%) and protein (>30%) proportions for commercial dietary formulation, analyses indicate that FA composition and amount play a key role in *A. astacus* growth, particularly in RAS. At least 15 measured FAs are required in an appropriate crayfish diet in sufficient amounts to achieve high growth rates while not losing energy and growth potential for FA synthesis. The FA profile of *Elodea* spp. and *M. edulis* indicate suitability as a feed or at least as a viable supplementary dietary component.

KEY WORDS: *Astacus astacus*, crayfish feed, fatty acid profile, lipid content, recirculating aquaculture system, open pond system

INTRODUCTION

The noble crayfish *Astacus astacus* is native to nearly all freshwater systems in Europe (Ingle 1997, Westman 2002) and was previously a common human food source (Cukerzis 1988, Skurdal & Taugbøl 2001, Holdich 2002). After the introduction of the American crayfish species, *Pacifastacus leniusculus* and *Orconectes limosus*, during the late 19th century and concomitant highly infectious crayfish plague (*Aphanomyces astaci*) *A. astacus* numbers in Europe decreased drastically (Westman & Savolainen 2001, Edgerton et al. 2002, Holdich 2002, Holdich et al. 2010). Other factors, such as water pollution, habitat alteration and destruction, have led to the species now being considered endangered (Edsman et al. 2010, Gherardi 2011) with only small and isolated wild populations remaining (Füreder 2009). Limited availability has increased the economic value to that of a luxury food product available only in small quantities on local markets for currently 35–50€/kg live weight (Franke et al. 2011).

The noble crayfish is of commercial interest for aquaculture and for restocking measures, including production in recirculating aquaculture systems (RAS), but effectively formulated diets are lacking to reach commercial production levels. Its high meat content and quality, along with high economic value make *Astacus astacus* a desirable culture species. Currently, crayfish culture is conducted in open pond systems (OPS) which have several disadvantages such as risk of infection, long time to harvest, and high workload, whereas crayfish are fed on a natural diet (Cukerzis 1988, Ackefors 2000). As already

proposed in other studies, RAS offer more stable and economically viable conditions in general (Lawson 1995) and specifically for crayfish production (Franke et al. 2011, Seemann et al. 2014) by excluding the risk of infection, compared with OPS and reducing seasonal growth variation. Understanding of total lipid and essential fatty acid (FA) requirements is therefore key to diet formulation for successful on-growing.

Lipids and FA composition play a major role in forming structures for growth and in the metabolism of living organisms, especially providing the energy needed for physiological activities. Even if crustaceans are able to generate essential FAs on their own in considerable amounts (Zandee 1962), the ratio and quantity of supplied lipids via feed directly influence the amount of energy and resources available for growth (Araujo & Lawrence 1991, Teshima et al. 1992, Cahu et al. 1995). Lipid composition can be affected by biotic factors such as crayfish age, sex, reproductive cycle, and abiotic factors such as climate (Farkas & Nevenzel 1981, Huner et al. 1990, Dey et al. 1993).

Reported feed requirements for freshwater crayfish in general include a lipid content (LC) of about 10% and a protein content of 25%–30% (Jover et al. 1999, Valipour et al. 2012, Xu et al. 2013). These ratios are also suggested for *Astacus astacus* (Ackefors et al. 1992), but no further specifications are reported and no commercial extruded crayfish diets are available. The basic nutritional requirements outlined earlier are, however, supplied by various commercial carp feeds. Seemann et al. (2014) found pelleted carp feed insufficient for commercially viable noble crayfish growth in RAS (Fig. 1). The specific growth rate (SGR) in RAS (RAS HI SGR 0.78%) was significantly (0.45%) lower than in OPS (SGR 1.23%). Whereas energy content (EC) of the feed was

*Corresponding author. E-mail: uli.seemann@awi.de
DOI: 10.2983/035.036.0223

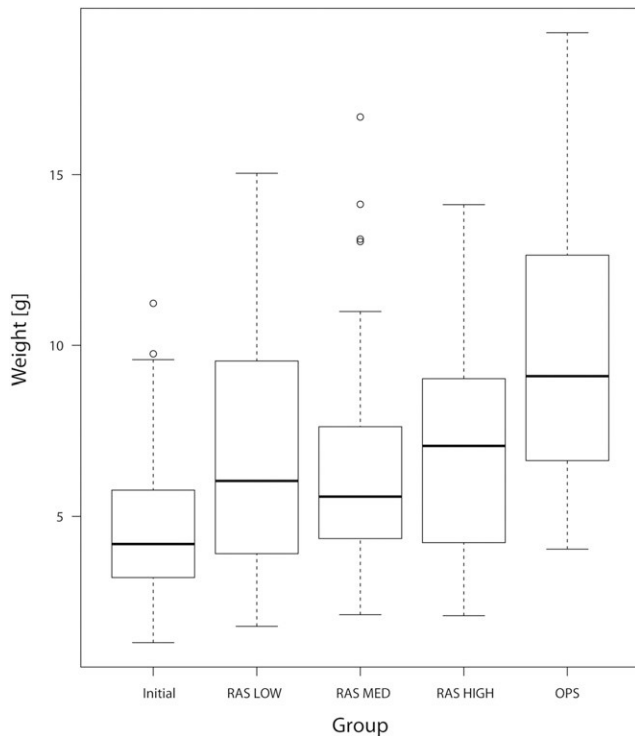


Figure 1. Weight of treatment groups OPS (not fed), Initial sample, and three RAS measured at the end of the experiment. RAS LOW (3%), MED (4%), and RAS HIGH with $n = 20$, OPS with $n = 20$, and Initial with $n = 90$. Whiskers indicate 1 SD. Black line in each box represents the median value.

sufficient (16.95 MJ kg^{-1}), water stability, structure as well as protein amount, and FA composition were named as possible reasons for the poor growth performance.

Meal of the blue mussel (*Mytilus edulis*) and waterweed (WW) (*Elodea* spp.) have been identified as alternative feed resources and potential diet components to improve *Astacus astacus* feed, as mussels and WW belong to the natural food spectrum (Huner 1994, Hager 2003, Füreder 2009). In addition, both species accumulate in vast amounts either on foundations of offshore wind farms and several other artificial reefs (*M. edulis*) or in lakes and ponds (*Elodea* spp.). These structures and water bodies have to be kept clean by regular maintenance activities at high costs. Reusing these resources would be sustainable and economically feasible, especially in view of continuously rising fish meal and fish oil prices (Weiß 2013, Nagel et al. 2014, Weiß & Buck 2017). The energy and LCs as well as the FA profiles of these species were investigated to give conclusions about their suitability as feed supplements or ingredients.

To investigate why *Astacus astacus* fed commercial carp feed in RAS exhibit poorer growth performance than those in OPS (where crayfish relied only on natural foraging) and to draw conclusions about a suitable formula and diet for RAS crayfish, this study aimed to examine

- The FA profile of noble crayfish reared in two different systems with regard to growth performance (RAS, pond)
- The feasibility of using *Mytilus edulis* and *Elodea* spp. as alternative feeds or feed ingredients in a noble crayfish diet reared in a RAS

MATERIALS AND METHODS

Experimental Design

A total of 99 summerling crayfish (14-mo-old) were collected from a commercial crayfish OPS farm (Poggenhagen, Germany) on June 28, 2012 and transported to the Center for Aquaculture Research (Bremerhaven, Germany) for use in a controlled feeding experiment in an RAS (Seemann et al. 2014). Nine crayfish were sampled whole body for tissue on collection, analyzed for FA amounts and composition and served as an initial value. The remaining 90 crayfish were maintained in replicate ($n = 3$ per treatment) tanks in an RAS at a density of 28 individuals per m^2 and fed a pelleted, nonfloating carp feed [Cyprinin K2 (CK2), Muskator Werke, Düsseldorf, Table 1] at three daily rations, defined as diet treatments as follows:

3% of body weight = RAS LOW

4% of body weight = RAS MED

5% of body weight = RAS HIGH

Trials were in triplicate over a period of 60 days. At the end of the experimental feeding period, three animals per replicate tank were sampled as well as nine crayfish in parallel from the OPS at the end of the experiment for FA analysis. The OPS crayfish relied only on natural foraging. After completion of the feeding trial, the SGR (SGR in % d⁻¹) was determined as follows:

$$\text{SGR} = 100 * (\text{LN}(\text{final weight}) - \text{LN}(\text{initial weight}) / \text{time interval}) \quad (1)$$

Fatty Acid Analysis

Sampling

Samples ($n = 9$) were taken from OPS and RAS to determine the whole-body FA composition of *Astacus astacus* at the end of the experiment. After determining the size and weight, the animals were placed into 50-ml falcon tubes, frozen in liquid nitrogen and stored until further processing at -80°C . The samples were then dried in a freeze dryer (Alpha 1-4 LSC; Christ, Osterode am Harz, Germany) at 0.2 mbar to constant weight. Using a mortar and pestle, the crayfish were individually ground to powder. The mussel meat meal (MM) was prepared from *Mytilus edulis* collected nearshore in the Wadden Sea at Wilhelmshaven (Germany) in May 2012 and *Elodea* spp. (WW) was collected from the crayfish farm ponds (Poggenhagen, Germany). The MM was produced by removing the meat from the shell, discarding the shell and drying at 65°C in a drying oven (Heraeus oven, Thermo Fisher Scientific, Germany). The same drying protocol was used for the WW whole plant samples. Mussel meal, WW, and CK2 were then ground in a mortar for further processing. The FA profiles of CK2, WW, and MM were determined (double-determination) from triplicate samples.

Lipid Extraction

Lipid extraction was carried out following Folch et al. (1957) as modified by Koch (2012): a total 0.1 g of the sample was weighed in a 50-ml beaker, and 100 μl of internal standard was added. Then, 6 ml of dichloromethane (DCM)-methanol

TABLE 1.

Manufacture's information about the ingredients of the used carp feed CK2, Muskator company, Düsseldorf, Germany.

Grain size (mm)	2.0
Raw protein (%)	25.0
Raw lipid (%)	6.8
Raw fiber (%)	6.4
Raw ash (%)	6.9
Calcium (%)	1
Gross energy (MJ kg ⁻¹)	14.8

(MeOH) mixture, (2:1) (MeOH/DCM) was added, and the sample was homogenized in an ultrasonic bath for 10 min. Next, the sample was transferred to a separating funnel and the beaker rinsed twice with 3 ml of MeOH/DCM. Five milliliters of 0.88% KCl solution was added. The sample was shaken for 2 min and vented multiple times. After the addition of 5 ml of DCM, the sample was agitated, and after separation, the lower phase was drained into a conical flask. During the reprocessing of the crayfish samples, a third phase was apparent in the separating funnel, which did not separate into solvent and aqueous phase. Therefore, 10 ml DCM instead of 5 ml was added after the addition of KCl which improved the separation significantly. The aqueous phase was re-extracted twice with 5 ml of DCM and the extract collected in the conical flask. The solvent was then removed in a rotary evaporator (BÜCHI 011 Style Rotovapor, BÜCHI Labortechnik, Essen, Germany) under vacuum. The remaining lipids were transferred with DCM using a Pasteur pipette into a balanced Schott flask, and the solvent was evaporated again with N₂. The total LC could be determined by reweighing. Then, the addition of 500 µl of n-hexane and 2,000 µl derivatization reagent (50 ml MeOH and 1.5 ml sulfuric acid) followed. The sample was placed overnight in a heating block, maintained at a temperature of 70°C for 4 h and then cooled to room temperature (21°C). Then, 4 ml of ultrapure water was added and the whole extracted with 3 ml of n-hexane. The n-hexane phase was pipetted into a conical flask. This process was repeated two times with 2 ml n-hexane. In a rotary evaporator, the solvent was removed, and the lipids were transferred with 1 ml of n-hexane in a balanced gas chromatograph (GC) vial. The total ester content was determined by evaporation with N₂ and weighed again. The lipids were dissolved in 1 ml of n-hexane and were available for further analysis with the GC. Owing to their liposolubility, carotenoids were extracted within the lipid extraction analysis.

Fatty Acid Analyses Via Gas Chromatography

For GC processing, the extracted lipids were diluted 1:5. An internal standard 23:0 FAME standard (Sigma Aldrich, Germany) was prepared by dissolving 5 ml n-hexane in 25 mg methyl tricosanoate. To determine the recovery rate, the FAME 23:0 was added at a 1:10 dilution (50 ng µl⁻¹) as an injection standard on the GC. To identify the individual components, the FAME-Mix-Standard (Supelco 37) was used with a concentration of 1 mg ml⁻¹ after Sigma-Aldrich (2008). A run with n-hexane and the two standards was completed on each measurement on the day before injecting samples into the GC. The GC unit was a Chrompack CP 9002. The

starting temperature was 150°C and the final temperature 280°C. The column used was DB-FFAP (30 m × 0.25 × mm × 0.25 µm), Agilent Technologies (Böblingen, Germany), and the carrier gas used was helium. The injected volume of the sample was 1 µl. The software used, Clarity™ 4.0 DataApex (Praha, Czech Republic), had an automatic peak detection area. In addition, the chromatograms were manually examined for any misinterpretation.

Quantification of Fatty Acids

After identifying the peaks, the FAs were quantified with the internal standard (FAME 23:0). The calculation of the weight of the FA was made using Eq. (1).

$$W_{FA} = \frac{A_{FA}}{A_{Inj.Std.}} \cdot GW_{Inj.Std.} \quad (2)$$

W_{FA} = weight of the FA in the sample (ng)

A_{FA} = peak area of the FA in the sample

A_{Inj.Std.} = peak area of the FA in the injection standard

G_{Inj.Std.} = total weight of the FA in the injection standard FAME 23:0 (ng)

Subsequently, the conversion into the weight percentages was done using Eq. (2)

$$WP = \frac{G_{FA}}{G_{FA\ total}} \cdot 100\% \quad (3)$$

WP = weight percentages of the FA in the sample (%)

G_{FA} = weight of the FA in the individual sample

G_{FA total} = total weight of the quantified FA in the sample (ng)

Crayfish samples in all treatments were analyzed separately in relation to each FA. The FAs C14:0, C20:1n9, and C22:6n3/C24:1n9 were detected only once or twice in the samples; nevertheless, they have been listed. Five samples could not be analyzed because of low recovery rate, particles in the extract or nonesterification. The FAs C18:1n9c/C18:1n9t, C18:2n6c/C18:2n6t, and C22:6n3/C24:1n9 could not be separated because of an overlap of peaks.

Calorimetry

The EC of the CK2, MM, and WW were determined by calorimetry. Samples were dried at 65°C in a drying oven Heraeus oven (Thermo Fisher Scientific, Germany), ground in a mortar, and weighed with a precision scale to ±0.1 mg (Sartorius CPA224S). A pellet was pressed from the powder and weighed again. A calorimeter (6,100 Calorimeter, Parr Instrument Company) was used for further processing with the following specifications of bomb no.1 (Cap 41A 394A18 052 109, cylinder 101A C20 063 009 M26063). The bucket was filled with 2,000 ± 0.5 g deionized water. The pellet was placed into the combustion chamber of the bomb and attached to the wire. Then the bomb was sealed, filled with oxygen, and placed in the calorimeter. The measurement was automatically done after entering the sample weight; the result was displayed in MJ kg⁻¹. Of each sample, a triplicate measurement was done.

Statistics

Statistical analysis was performed using the R program (Version 2.10.1.) The experimental treatments were tested with a Shapiro–Wilk test for normal distribution and with a Bartlett test for homogeneity of variance. Treatments were compared via *t*-test or analysis of variance (ANOVA). Significant differences in mean values between two RAS treatments were located using a Tukey's *post hoc* test. A Kruskal–Wallis test and the pairwise Wilcoxon test were performed to locate significant differences between the experimental treatments when normal distribution or homogeneity of variance was not given. The size and weight data and the growth rates between indoor and outdoor feeding trials were compared by a one-sample *t*-test with a confidence interval of 95%. Values that were marked as outliers by R were not considered in further calculations. The software considers outliers as values located more than 1.5 times from the interquartile range. This boundary also marks the maximum possible extent of the plot whiskers. For the FA profile, a principal component analysis (PCA) was performed alongside the ANOVA to better visualize differences in FA composition between the treatments. The PCA used the means of FAs to offer an insight into similarities via the locations of treatments. The arrows of the individual FAs indicate their individual influence on the location of the treatments. Because of errors in the analytical measurements (LC, EC), the replicate numbers of treatments RAS HIGH and OPS had to be lowered to $n = 5$ and $n = 8$, respectively.

RESULTS

Lipid and Energy Content

The mean LC of the crayfish tissue dry weight differed significantly between treatments (Kruskal–Wallis test, $df = 4$, $\chi^2 = 18.5$, $P < 0.01$; Fig. 2). Crayfish from the pond system $8.51 \pm 2.13\%$ exhibited significantly higher LC after 8 wk than crayfish in all RAS treatments (Wilcoxon test, RAS LOW versus OPS $P < 0.01$; RAS MED versus OPS $P < 0.05$; RAS HIGH versus OPS $P < 0.01$). Mean tissue LC content did not differ significantly between RAS treatments 5.73 ± 1.66 (RAS LOW), $6.93 \pm 1.72\%$ (RAS MED), and $5.92 \pm 1.54\%$ (RAS HIGH).

Change in mean tissue LC content over the experimental period (Initial value of all treatments = $9.67 \pm 2.21\%$) was $-2.78 \pm 1.66\%$ (RAS LOW), $-1.58 \pm 1.72\%$ (RAS MED), $-2.59 \pm 1.54\%$ (RAS HIGH), and $+1.06\% \pm 2.21$ in OPS crayfish. Lipid content of the feed ranged from 4.1% to 4.5% and mean EC was 16.95 ± 0.11 MJ·kg⁻¹·dw. Lipid content of MM was 8.9% and the mean EC 15.87 ± 0.15 MJ·kg⁻¹·dw. Lipid content of WW was 8.5% and the mean EC 14.70 ± 0.10 MJ·kg⁻¹·dw (Table 2).

Fatty Acid Profiles

Fatty acid profiles differed significantly between RAS and Initial/OPS treatments but did not differ significantly between RAS treatments.

The FAs C16:0, C18:1n9c/C18:1n9t, and C18:2n6c/C18:2n6t each contributed more than 20% of total lipids (Table 3). Initial samples showed significantly lower relative concentration of compound C18:2n6c/C18:2n6t ($14.84 \pm 1.83\%$)

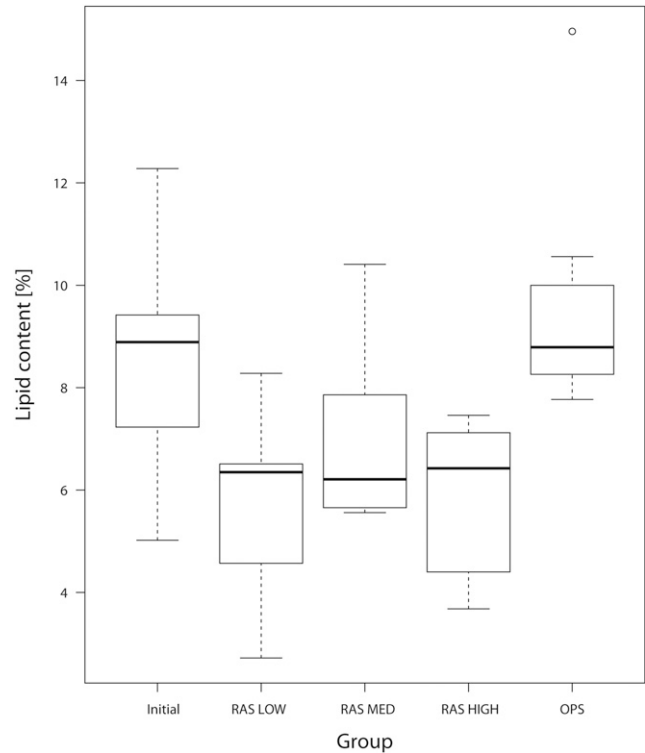


Figure 2. Treatment groups consist of OPS (not fed), Initial sample, and three RAS treatments fed various levels of a commercial carp diet (CK2) based on % body weight. RAS LOW (3%), MED (4%), and Initial with $n = 9$, RAS HIGH (5%) with $n = 5$, and OPS with $n = 8$. Whiskers indicate 1 SD. Black line in each box represents the median value.

compared with all other treatments. Significant differences between treatments were found for FAs C16:1, C18:0, C18:2n6c/C18:2n6t, C18:3n3, C20:3n3, and C20:5n3 (ANOVA or Kruskal–Wallis), whereas the Initial treatment showed most significant differences against all other treatments (Tukey's *post hoc* test or pairwise Wilcoxon test).

Repeated determination of FA analysis of CK2 showed a domination of C18:2n6c/C18:2n6t (omega 6, linoleic, and linolelaidic) with $43.62\% \pm 0.50$. The lowest proportion was FA C18:0 (stearic) with $3.85\% \pm 0.67$. C16:0 (palmitic) and omega 9 C18:1n9c (oleic) and C18:1n9t (elaidic) ranged between 11%–15%. Oleic and elaidic were only found in CK2.

TABLE 2.

Energy content and LC of feed, waterweed, and MM.

Sample	Energy	
	Mean \pm SD (MJ kg ⁻¹ dm)	Lipid (%)
CK2	16.95 ± 0.11	4.1/4.6
<i>Elodea</i> spp.	14.70 ± 0.10	8.5
<i>Mytilus edulis</i>	15.87 ± 0.15	8.9

Energy content and LC of commercial carp diet (CK2, $n = 2$), MM (*Mytilus edulis*), and *Elodea* spp. ($n = 3$) based on samples of dry weight.

TABLE 3.
Fatty acid composition per group.

FA	Weight proportion of FA per group (%)					P-value
	RAS LOW	RAS MED	RAS HIGH	Initial	OPS	
C14:0	–	–	–	–	0.46 ± 0.86	0.0844 (KW)
C16:0	22.70 ± 2.94	22.63 ± 2.77	22.32 ± 2.06	19.82 ± 3.37	22.66 ± 1.72	0.1399 (ANOVA)
C16:1	3.33 ± 2.04	4.25 ± 1.83	5.02 ± 1.20	4.79 ± 2.17	6.37 ± 1.36	*0.0156 (KW) (OPS – 3% and 4%)
C18:0	8.07 ± 2.10	6.64 ± 2.65	6.51 ± 1.47	7.51 ± 1.47	5.99 ± 0.96	*0.0299(KW) (3%–4%, Initial – 4%, OPS – Initial)
C18:1n9c/C18:1n9t	24.07 ± 2.46	25.78 ± 2.28	24.08 ± 3.25	23.40 ± 7.60	20.22 ± 2.74	0.0697 (KW)
C18:2n6c/C18:2n6t	27.29 ± 2.82	28.26 ± 1.83	27.47 ± 5.06	14.84 ± 1.83	23.54 ± 2.09	*3.1 × 10 ⁻¹² (ANOVA) (Initial – 3%, 4%, 5% & OPS)
C18:3n3	3.014 ± 2.49	3.85 ± 2.26	4.29 ± 0.82	9.04 ± 2.19	9.79 ± 2.59	*9.5 × 10 ⁻⁶ (KW) (Initial – 3%, 4%, 5%, OPS – 3%, 4%, 5%)
C20:1n9	–	–	0.49 ± 1.10	–	–	0.1359 (KW)
C20:2	1.05 ± 1.59	0.74 ± 1.15	1.32 ± 1.93	0.33 ± 0.99	–	0.3032 (KW)
C20:3n3	4.85 ± 3.31	3.58 ± 1.59	4.63 ± 3.01	9.15 ± 3.97	4.56 ± 1.16	*0.0027 (KW) (Initial – 3%, 4%, 5% & OPS)
C20:5n3	5.47 ± 2.66	4.08 ± 1.82	3.87 ± 3.57	10.85 ± 2.30	6.42 ± 1.53	*2.9 × 10 ⁻⁶ (ANOVA) (Initial – 3%, 4%, 5% & OPS)
C22:6n3/C24:1n9	–	0.18 ± 0.54	–	0.29 ± 0.87	–	0.6429 (KW)

Fatty acid composition of the lipid portion of whole crayfish (*Astacus astacus*) in OPS and RAS fed a commercial carp diet (CK2). Mean values given are based on the percentage of dry weight. Group 3%, 5% and start $n = 9$, 5% $n = 5$, pond end $n = 8$. P values were calculated by ANOVA or Kruskal–Wallis test (KW). Marked (*) groups show significant differences.

C16:0 proved to be the dominant FA in MM with a weight proportion of 31.87% ± 0.05%, followed by C20:5n3 28.01% ± 0.34% (Table 4). The dominant FA in WW was C18:3n3 with 54.72% ± 0.66%. C16:1 was detected in low quantities (4.11% ± 0.22%). Identified FA C18:2n6c/C18:2n6t and C18:3n3 in the WW could not be detected in MM, whereas C14:0, C18:0, C20:3n3, C20:5n3, and C22:6n3 were detected in the MM but not in the WW.

Principal Component Analysis

The principal components 1 and 2 together account for 82% of the variation in FA profile data (Fig. 3). Treatments 3, 4, and

5% all display in close vicinity, whereas treatments Initial and OPS each are clearly located in different areas of the biplot. As FA C18:3n3 is significantly higher in treatments Initial and OPS than in RAS treatments (Table 3), its influence on these two treatments is higher accordingly, resulting in a shift and separation of peaks. On the other hand, FA compounds C18:1n9c/C18:1n9t and C18:2n6c/C18:2n6t showed higher amounts in RAS treatments and led to a separation of treatments Initial and OPS.

DISCUSSION

Commercially viable aquaculture of *Astacus astacus* requires formulated diets suited to the animal's nutritional requirements for optimal growth. Recent results showed that noble crayfish fed with a common carp diet did not perform as well as crayfish in OPS (Seemann et al. 2014). In this study, FA analyses of RAS and OPS crayfish were conducted to give information about the feed requirements of these crayfish. Determining the suitability of EC and lipid profile in proposed diets is essential to culture optimization. The current study attempted to address this by determining the FA profiles of proposed formulated diet (CK2) and natural constituents suggested as diet components in the form of *Elodea* spp. (WW) and MM from *Mytilus edulis* which is a common fouling organism in the North Sea and thus comparatively readily and cheaply available (Weiß & Buck 2017).

The proposed crayfish diets CK2 (16, 95 ± 0, 11 MJ kg⁻¹ dm), MM (15, 87 ± 0, 15 MJ kg⁻¹ dm), and WW (14, 70 ± 0, 10 MJ kg⁻¹ dm) exhibited similar ECs to that quoted in the literature (Fredrickson & Heitmeyer 1991, Beukema 1997, D'Agaro et al. 2004) indicating the potential of these resources as feed ingredients in terms of EC, even if EC amounts may vary depending on the location-based conditions. In a previous

TABLE 4.

Composition of FAs in commercial diet and alternative feeds.

FA	Mean proportion of weight of FAs (%)		
	CK2	MM	WW
C14:0	–	3.89 ± 0.37	–
C16:0	16.62 ± 0.51	31.87 ± 0.05	19.77 ± 0.16
C16:1	–	13.18 ± 0.07	4.11 ± 0.22
C18:0	3.85 ± 0.67	7.77 ± 0.14	–
C18:1n9c/C18:1n9t	24.79 ± 1.12	–	–
C18:2n6c/C18:2n6t	43.62 ± 0.50	–	22.33 ± 0.64
C18:3n3	11.13 ± 0.86	–	54.72 ± 0.66
C20:3n3	–	3.98 ± 0.14	–
C20:5n3	–	28.01 ± 0.34	–
C22:6n3	–	11.31 ± 0.34	–

Fatty acid composition of a commercial carp diet (CK2), MM (*Mytilus edulis*), and *Elodea* spp. (WW) analyzed by repeated determination ($n = 3$) of dry weight.

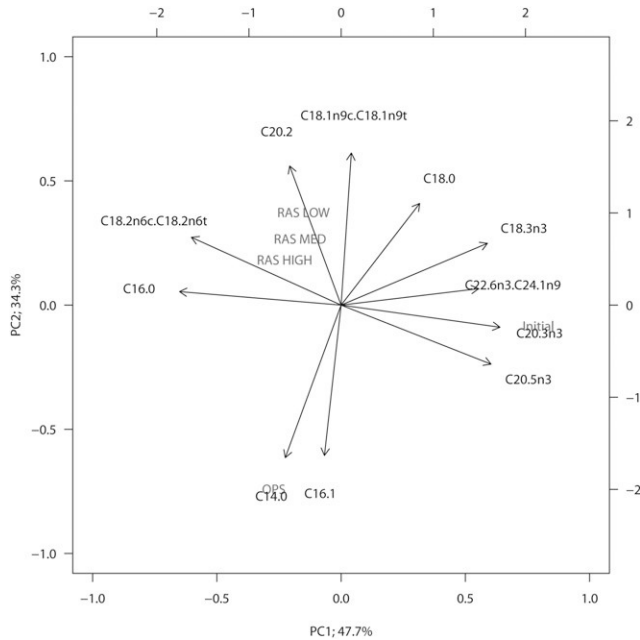


Figure 3. Biplot of the PCA-analysis of the FA composition for the experimental groups. For visualization, each crayfish sample and its location regarding the FA content proportion is displayed ($n = 40$). Clustering was added for better visualization.

study, no significant difference in SGR was found in RAS crayfish fed at different feed ratios; however, all RAS treatments had a significantly lower LC and poorer growth performance than OPS crayfish on the short term (Seemann et al. 2014). The LC in the CK2 feed ranged between 4.1% and 4.5%, which was below the manufacturer's reported levels of 6.8% but still were in the suitable range (<10%) for a crayfish diet reported by Ackefors et al. (1992). Given that considerable amounts of CK2 remained after feeding overnight in all treatments, more feed is unlikely to have resulted in higher LC and better growth performance of the crayfish. Results indicate a slightly higher LC amount in the natural forage available to OPS crayfish. Whether feed structure hampers handling and uptake or nutrient composition of the diet itself influences digestion and therefore are responsible for lower lipid accumulation in RAS crayfish remains unsettled. The reason for lower lipid accumulation in RAS crayfish remains unsettled. Whether feed structure hampers handling and consumption or nutrient composition of the diet itself influences digestion has to be clarified.

As shown by Rozentsvet et al. (1995), LC can vary in macrophytes between 8.8% and 17.8% of dry matter depending on the environmental conditions and harvest time. Fredrickson and Reid (1988) and D'Agaro et al. (2004) measured lower LC for other water plants such as *Elodea Canadensis* Michx. resulting in growth decrease if used as a single feed. The analysis showed 8.5% for WW which is within the recommended range <10% (Ackefors et al. 1992, D'Agaro et al. 2004). The LC of MM (8.9%) is confirmed by the literature and would fit to the requirements of a crayfish diet. Beukema (1997) stated values between 4%–12%, depending on season and mussel conditions. In combination with MM, no negative growth effects caused by too low lipid levels are to be

expected. Amounts of MM in a feed also offer the possibility to adjust the protein content in a given range and reducing feeding intervals while maintaining constant growth performance (Pasini 2013). In summary, using WW and MM as part of a formulated diet or a feed component would be possible, but both have to be preprocessed to pass restrictions for feed production in the European Union.

The FAs C14:0, C20:1n9, and C22:6n3/C24:1n9 were detected once or twice in low amounts in crayfish and not in the diet in the current study but were reported by Ackefors et al. (1997) at higher rates in crayfish, indicating that these FAs were insufficient in the crayfish diet but could be essential for crayfish performance. The essential FAs for crustaceans linoleic acid (18:2n-6) and linolenic acid (18:3n-3) (Wickins & Lee 2002) were supplied by the diets whereas eicosatrienoic acid (ETE) (C20:3n3), eicosapentenoic acid (20:5n-3), and docosahexaenoic acid (DHA) (22:6n-3) were completely absent in CK2. Although linoleic acid was measured without significant differences in OPS and RAS crayfish and was available in high amounts in CK2, linolenic acid had a significantly lower proportional weight in RAS than in OPS crayfish. Therefore, linolenic acid supplied by CK2 appears to be insufficient as indicated in the PCA. Eicosatrienoic acid (C20:3n3) was not included in CK2 but measured in considerably high amounts in all crayfish with significantly highest rates at the experimental beginning. This indicates that FA composition changes with crayfish age and size and gives evidence of the potential of crayfish to synthesize FA on their own as stated by Zandee (1966) and Teshima et al. (1992). Nevertheless, ETE should be included in a crayfish diet in a high proportion to minimize efforts of crayfish to synthesize FAs. The FA C22:6n3 (DHA) is essential for crustaceans, particularly for juveniles (Guillaume 2001) and can be synthesized in small but insufficient quantities. Here, DHA as well as arachidonic acid (20:4n-6) were neither part of the feed nor found in crayfish analyzed in the present study but detected by Ackefors et al. (1997) in tail tissue of *Astacus astacus*. Two FAs [eicosatrienoic acid (20:3n-3), C20:2] measured in RAS and Initial were not found by Ackefors et al. (1997) and OPS samples. Missing FAs could have caused slower growth in OPS crayfish even if OPS performance was better than in RAS and indicate potential for OPS feed and growth improvement.

Significant differences in other FA concentrations between RAS and OPS/Initial treatments could also be caused by CK2. The main FA mix (C18:2n6c/C18:2n6t) in CK2 proved to be significantly higher in RAS crayfish and led to a separation from RAS groups in the PCA (Fig. 3). The FA mix C18:1n9c/C18:1n9t also showed higher amounts in RAS crayfish possibly because of its main share in CK2 and facilitated the separation in the PCA from RAS groups. It can be assumed that these FAs were at least oversupplied. Negative effects such as decreased growth in RAS crayfish could not be negated as well. By contrast, FA C18:3n3 was significantly lower in RAS (9.84% and 12.20%) than in OPS where the foraged WW supplied high amounts of this FA (55.71% and 54.14%) and may have led to better growth in OPS.

Significantly higher amounts of C18:2n6c/C18:2n6t in OPS than in the Initial treatment can be explained by the better capability of the older and double-sized crayfish

(Seemann et al. 2014) to handle resources like the foraged and widespread WW with high amounts of these FAs. Younger and smaller crayfish cannot grab and break WW apart and prefer feeding on easy-to-handle resources such as tender shoots (Skurdal & Taugbøl 2001, Wittmaack 2006) which is generally valid for *Astacus astacus* (Hager 2003). A change in the metabolism of natural forage could also influence its FA composition and therefore the FA composition of the feeder like stated e.g. for grass carp (Cai & Curtis 1990, Venkatesalu et al. 2012). Because daily climate conditions were mostly consistent over the experimental period, such effects would have had a minor effect. Regarding the season, quantities of feed resources were stable in the summer season and over the 2-mo experimental period. Although in RAS, climate conditions were unified all over and significantly higher amounts of C18:2n6c/C18:2n6t, in RAS, crayfish can be related to food source CK2 which comprise a very high amount of these FAs.

Differences between Initial and OPS can be explained by different feed-handling capabilities with increasing crayfish size, a change in feed and nutritional requirements, or a varying food supply in the pond (Pöckl 1998). A shift in the feed requirements and capabilities is shown by the significant results of lower amounts of FA C20:3n3 and C20:5n3 in older crayfish (OPS) than in younger crayfish (Initial). Although it is reported by Skurdal and Taugbøl (2001) that *Astacus astacus* juveniles prefer benthic invertebrates rather than plants, survival and SGR of crayfish fed with a diet mix of pellets and American WW (*Elodea canadensis* Michx.) showed the best performance compared with a pelleted diet and a WW diet (D'Agaro et al. 2004). Protein content of this pellet carp feed was 40.9% and corresponds to the recommendation for juveniles (Ackefors et al. 1992).

Waterweed has high amounts of linolenic acid and could improve the FA quantity if included in a formulated crayfish feed. The essential FAs eicosapentenoic acid and DHA are absent in both CK2 and WW and could be supplied in combination with MM. Adding MM with amounts of 70% protein (Nagel et al. 2014) would also partly enhance the relatively low protein content of WW 25%–35% (D'Agaro et al. 2004). The bottom line is that the combination of CK2, MM, and WW would cover the FA profile of *Astacus astacus* and its FA demand better than CK2 alone. Variances in WW and MM composition can vary dependent on collection site and time of collection and must be accepted and accounted for. Offshore collected mussels e.g., could vary in FA composition from the nearshore collected mussels which were used in this study.

Regarding the FA amounts in RAS crayfish, it has to be taken into account that only seven of 15 (12) FAs (partly combined) measured in crayfish were supplied by the feed. Considering the FA profiles of all treatments, MM and WW would be potential feed additions for CK2 or can be used as an exclusive feed. A mix of CK2, MM, and WW would supply 12 of 15 FAs detected in crayfish whereas MM and WW alone provide nine (10) of these FAs. C20:2 and two omega 9 FAs, C20:1n9 and C24:1n9 (nervonic) would have to be implemented.

The overall protein and fiber content could be varied by WW and MM ratio in relation to the crayfish demands and

age. Water weed would provide carotenoids and ensure coloration as well as other positive effects on growth and health (Thacker et al. 1993, Guillaume 2001, Kristiansen et al. 2004). D'Agaro et al. (2004) as well as Geddes and Smallridge (1993) obtained the same results regarding the potential of *Elodea canadensis* as a supplemental feed for noble crayfish where best growth performance was achieved with a dietary mix of pellets and plants. As the most expensive ingredients in feed production are fishmeal proteins, low-cost protein resources and alternatives like these secondary raw materials become more important and are already used in aquaculture (Geddes & Smallridge 1993, Jones et al. 2002, D'Agaro 2006, Fisheries 2013, Fuertes et al. 2013). Fuertes et al. (2012) concluded that for juvenile crayfish *Pacifastacus leniusculus*, up to 25% of fishmeal could be replaced with soybean meal. Whether it needs a preprocessing of WW and MM depends on the origins of resources, the farm system and culture conditions, and the given restrictions for food production but would be mandatory for enclosed and intensive systems (Wiernicki 1984, D'Agaro et al. 2004, D'Agaro 2006). To what extent the nutrient requirements and therefore feed composition change with the crayfish age must be analyzed in further studies.

CONCLUSION

The noble crayfish is an omnivorous but specific feeder focusing on invertebrates, plants, and detritus (Skurdal & Taugbøl 2001). For *Astacus astacus* held in RAS or other high-intensity culture, the provided feed and its ingredients play a crucial role in growth performance. Whereas Ackefors et al. (1992) determined lipid (<10%), carbohydrate (20%–25%), and protein (25%) proportions for commercial diet formulation for young crayfish, the effect of the FA as well as amino acid levels and compositions also play an essential role in achieving comparable or better growth rates to common production techniques (OPS).

The current results suggest that both *Mytilus edulis* and *Elodea* spp. can be used in crayfish production as a basis for a pelleted, extruded feed, or at least as a supplementary diet. The results generated in the present study should be considered in crayfish dietary formulation for further studies. Different FA compositions would give insight into the best composition and whether they are essential. The importance of amino acid levels and compositions should also be examined to complement these outcomes. Finally, an economic study is necessary to evaluate diet production costs of a commercial diet incorporating WW and MM.

ACKNOWLEDGMENTS

We thank the reviewers for their time and thorough reviews which have significantly improved the manuscript. We also thank Kerstin Klockgeter, Claudia Burau, Stefan Wittke, and Boris Koch for their significant support, infrastructure, and resources for fatty acid analysis. We thank Monika Weiß and Lene Ennen for production and supply of the mussel meat meal. The study was supported by grants from the Deutsche Bundesstiftung Umwelt (German Federal Environmental Foundation, AZ 28879).

LITERATURE CITED

- Ackefors, H., J. D. Castell, L. D. Boston, P. Rätty & M. Svensson. 1992. Standard experimental diets for crustacean nutrition research. II. Growth and survival of juvenile crayfish *Astacus astacus* (Linné) fed diets containing various amounts of protein, carbohydrate and lipid. *Aquaculture* 104:341–356.
- Ackefors, H., J. Castell & I. L. Ördö-Öström. 1997. Preliminary results on the fatty acid composition of freshwater crayfish, *Astacus astacus* and *Pacifastacus leniusculus*, held in captivity. *J. World Aquacult. Soc.* 28:97–105.
- Ackefors, H. 2000. Freshwater crayfish farming technology in the 1990s: a European and global perspective. *Fish and Fisheries* 1:337–359.
- Araujo, M. A. & A. L. Lawrence. 1991. Fatty acids of muscle and hepatopancreas of juvenile *Penaeus vannamei* after non-terminal starvation and subsequent feeding with semi-purified diets: evaluation of fatty acid synthesis capabilities. *J. World Aquacult. Soc.* 22:13A.
- Beukema, J. J. 1997. Caloric values of marine invertebrates with an emphasis on the soft parts of marine bivalves: an annual review. *Oceanogr. Mar. Biol.* 35:387–414.
- Cahu, C. L., G. Cuzon & P. Quazuguel. 1995. Effect of highly unsaturated fatty acids, alphatocopherol and ascorbic acid in broodstock diet on egg composition and development of *Penaeus indicus*. *Comp Biochem Physiol A. Compr. Physiol.* 112:417–424.
- Cai, Z. & L. R. Curtis. 1990. Effects of diet and temperature on food consumption, growth rate and tissue fatty-acid composition of triploid grass carp. *Aquaculture* 88:313–327.
- Cukerzisz, J. M. 1988. *Astacus astacus* in Europe. In: Holdich, D. M. & R. S. Lowery, editors. Freshwater crayfish: biology management and exploitation. Cambridge, United Kingdom: Cambridge University Press. pp. 309–340.
- D'Agaro, E. 2006. Utilization of water cress (*Nasturtium Officinale* L.) in noble crayfish (*Astacus astacus*) feeding. *Bull. Fr. Peche Piscicult.* 380–381:1255–1260.
- D'Agaro, E., B. Renai & F. Gherardi. 2004. Evaluation of the American waterweed (*Elodea Canadensis* Michx.) as supplemental food for the noble crayfish, *Astacus astacus*. *Bull. Fr. Peche Piscicult.* 372–373:439–445.
- Dey, I., C. Buda, T. Wiik, J. E. Halver & T. Farkas. 1993. Molecular and structural composition of phospholipid membranes in livers of marine and freshwater fish in relation to temperature. *Proc. Natl. Acad. Sci. USA.* 90:7498–7502.
- Edgerton, B. F., L. H. Evans, F. J. Stephens & R. M. Overstreet. 2002. Synopsis of freshwater crayfish diseases and commensal organisms. *Aquaculture* 206:57–135.
- Edsman, L., L. Füreder, F. Gherardi & C. Souty-Grosset. 2010. *Astacus astacus*—status report. In: The IUCN Red List of Threatened Species: International Union for Conservation of Nature and Natural Resources. Available at: <http://www.iucnredlist.org/details/2191/0>.
- Farkas, T. & J. C. Nevenzel. 1981. Temperature-acclimation in the crayfish—effects on phospholipid fatty-acids. *Lipids* 16:341–346.
- Fisheries, G.-G. W. A.-D. o. 2013. Farming yabbies. In: Fisheries, D. o. editor. Perth, Western Australia: Government of Western Australia.
- Folch, J., M. Lees & G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 226.
- Franke, R., S. Wessels & G. Horstgen-Schwark. 2011. Enhancement of survival and growth in crowded groups: the road towards an intensive production of the noble crayfish *Astacus astacus* L. in indoor recirculation systems. *Aquacult. Res.* 44:451–461.
- Fredrickson, L. H. & M. E. Heitmeyer. 1991. Life history strategies and habitat needs of the northern pintail. *Fish and Wildlife Leaflet* 13.1.3:5–7.
- Fredrickson, L. H. & F. A. Reid. 1988. Nutritional values of waterfowl foods. In: Waterfowl Management Handbook. Columbia, Puxico, MO: University of Missouri.
- Fuertes, J. B., J. D. Celada, J. M. Carral, M. Saez-Royuela & A. Gonzalez-Rodriguez. 2012. Effects of dietary protein and different levels of replacement of fish meal by soybean meal in practical diets for juvenile crayfish (*Pacifastacus leniusculus*, Astacidae) from the onset of exogenous feeding. *Aquaculture* 364:338–344.
- Fuertes, J. B., J. D. Celada, J. M. Carral, M. Saez-Royuela & A. Gonzalez-Rodriguez. 2013. Replacement of fish meal with poultry by-product meal in practical diets for juvenile crayfish (*Pacifastacus leniusculus* Dana, Astacidae) from the onset of exogenous feeding. *Aquaculture* 404:22–27.
- Füreder, L. 2009. Flusskrebse Biologie, Ökologie, Gefährdung. Wien: Folio Verlag.
- Geddes, M. C. & M. Smallridge. 1993. Survival, growth and yield of the Australian freshwater crayfish *Cherax destructor* in extensive aquaculture ponds. *Aquaculture* 114:51–70.
- Gherardi, F. 2011. Towards a sustainable human use of freshwater crayfish (Crustacea, Decapoda, Astacidea). *Knowl. Managt. Aquatic Ecosyst.* 401:02.
- Guillaume, J. 2001. Nutrition and feeding of fish and crustaceans. London, United Kingdom: Springer.
- Hager, J. 2003. Edelkrebse, Biologie, Zucht, Bewirtschaftung. Graz, Stuttgart: Leopold Stocker Verlag.
- Holdich, D. M. 2002. Distribution of crayfish in Europe and some adjoining countries. *Bull. Fr. Peche Piscicult.* 367:611–650.
- Holdich, D. M., J. D. Reynolds, C. Souty-Grosset & P. J. Sibley. 2010. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowl. Managt. Aquat. Ecosyst.* 394–395:11.
- Huner, J. V. 1994. Freshwater crayfish aquaculture in North America, Europe, and Australia: families Astacidae, Cambaridae and Parastacidae. Boca Raton, FL: CRC Press.
- Huner, J. V., H. Kononen & O. V. Lindqvist. 1990. Variation in body-composition and exoskeleton mineralization as functions of the molt and reproductive-cycles of the noble crayfish, *Astacus astacus* L (Decapoda, Astacidae), from a pond in central Finland. *Comp. Biochem. Physiol. A* 96:235–240.
- Ingle, R. 1997. Crayfishes, lobsters and crabs of Europe—an illustrated guide to common and traded species. Dordrecht, The Netherlands: Springer.
- Jones, P., J. Chavez & B. Mitchell. 2002. Production of Australian freshwater crayfish in earthen-based systems using pelleted diets and forage crops as food. *Aquacult. Int.* 10:157–175.
- Jover, M., J. Fernandez-Carmona, M. Del Rio & M. Soler. 1999. Effect of feeding cooked-extruded diets, containing different levels of protein, lipid and carbohydrate on growth of red swamp crayfish (*Procambarus clarkii*). *Aquaculture* 178:127–137.
- Koch, B. 2012. Lipid extraction. Bremerhaven: University of Applied Science Bremerhaven, Marine Biotechnology Manuscript.
- Kristiansen, T. S., A. Drenstvig, A. Bergheim, T. Drenstvig, I. Kollsgard, R. Svendsen, E. Nostvold, E. Faresveit & L. Ardal. 2004. Development of methods for intensive farming of European lobster in recirculated seawater. In: Fischen og havet: Havforskning-sinstituttet. Bergen, Norway: Institute of Marine Research. p. 52.
- Lawson, T. 1995. Recirculating aquaculture systems. In: Fundam. of Aquac. Eng. New York, NY: Springer. pp. 192–247.
- Nagel, F., A. von Danwitz, M. Schlachter, S. Kroeckel, C. Wagner & C. Schulz. 2014. Blue mussel meal as feed attractant in rapeseed protein-based diets for turbot (*Psetta maxima* L.). *Aquacult. Res.* 45:1964–1978.
- Pasini, B. 2013. Growth of Nobel crayfish in RAS: suggestion for the protein and lipid content in a crayfish feed. Personal communication: ZHAW: Life Sciences und Facility Management, May 2013.
- Pöckl, M. 1998. Funktionelle morphologie und anatomie der flußkrebse. Austria: Linz: Biologiezentrum Linz. pp. 131–142.

- Rozenetsvet, O. A., N. M. Dembitsky & V. S. Zhuicova. 1995. Lipids from macrophytes of the middle volga. *Phytochemistry* 38:1209–1213.
- Seemann, U. B., K. Lorkowski, M. J. Slater, F. Buchholz & B. H. Buck. 2014. Growth performance of noble crayfish *Astacus astacus* in recirculating aquaculture systems. *Aquacult. Int.* 23:997–1012.
- Sigma-Aldrich. 2008. Fatty acid/FAME application guide—analysis of foods for nutritional needs. St. Louis, MO: Sigma-Aldrich. p. 24.
- Skurdal, J. & T. Taugbøl. 2001. Crayfish of commercial importance. In: Holdich, D. M., editor. *Biology of freshwater crayfish*. Oxford, United Kingdom: Blackwell Science. pp. 467–510.
- Teshima, S.-I., A. Kanazawa & S. Koshio. 1992. Ability for bioconversion of n-3 fatty acids in fish and crustaceans. In: Fauconneau & F. Takashima, editors. *Third France-Japan Conference in Oceanography on Growth Determinants in Aquaculture*. Oceanis. pp. 67–75.
- Thacker, R. W., B. A. Hazlett, L. A. Esman, C. P. Stafford & T. Keller. 1993. Color morphs of the crayfish *Orconectes virilis*. *Am. Midl. Nat.* 129:182–199.
- Valipour, A., R. O. A. Ozorio, F. Shariatmadari, A. Abedian, J. Seyfabadi & A. Zahmatkesh. 2012. Effects of dietary lipid levels on growth, survival, and molting of yearling narrow clawed crayfish, *Astacus leptodactylus*. *J. Appl. Aquacult.* 24:316–325.
- Venkatesalu, V., P. Sundaramoorthy, M. Anantharaj, M. Chandrasekaran & A. Senthilkuma. 2012. Seasonal variation on fatty acid composition of some marine macro algae from Gulf of Mannar Marine Biosphere Reserve, southeast coast of India. *Indian J. Geo. Mar. Sci.* 41:8.
- Weiß, M. 2013. Analyse der Nutzungsmöglichkeiten von biologischem Aufwuchs von künstlichen Hartsubstraten für die Gewinnung alternativer Protein- und Lipidressourcen. Bremerhaven: Bremerhavener Gesellschaft für Investitionsförderung und Stadtentwicklung mbH.
- Weiß, M. & B. H. Buck. 2017. Partial replacement of fishmeal in diets for turbot (*Scophthalmus maximus*, Linnaeus, 1758) culture using blue mussel (*Mytilus edulis*, Linnaeus, 1758) meat. *J. Appl. Ichthyol.* 33:354–360.
- Westman, K. 2002. Alien crayfish in Europe: negative and positive impacts and interactions with native crayfish. In: Leppäkoski, E., S. Gollasch & S. Olenin, editors. *Invasive aquatic species of Europe. Distribution, impacts and management*. Dordrecht, The Netherlands: Springer. pp. 76–95.
- Westman, K. & R. Savolainen. 2001. Long term study of competition between two co-occurring crayfish species, the native *Astacus astacus* L. and the introduced *Pacifastacus leniusculus* Dana, in a Finnish lake. *B. Fr. Peche Piscic.* 361:613–627.
- Wickins, J. F. & D. O. C. Lee. 2002. *Crustacean farming: ranching and culture*. Hoboken, NJ: Wiley-Blackwell.
- Wiernicki, C. 1984. Assimilation efficiency by *Procambarus clarkii* fed Elodea (*Egera densa*) and its products of decomposition. *Aquaculture* 36:203–215.
- Wittmaack, K. E. 2006. Analyses of nutritional ecology of the Noble crayfish (*Astacus astacus*), diet selection and prey experiments. In: Zoological Institute, Department Limnology. Kiel, Germany: Christian, Albrechts, University Kiel. p. 89.
- Xu, W. N., W. B. Liu, M. F. Shen, G. F. Li, Y. Wang & W. W. Zhang. 2013. Effect of different dietary protein and lipid levels on growth performance, body composition of juvenile red swamp crayfish (*Procambarus clarkii*). *Aquacult. Int.* 21:687–697.
- Zandee, D. I. 1962. Lipid metabolism in *Astacus astacus* (L.). *Nature* 195:814–815.
- Zandee, D. I. 1966. Metabolism in crayfish *Astacus astacus* (L.), IV. The fatty acid composition and the biosynthesis of the fatty acids. *Arch. Int. Physiol. Biochim.* 74:614–626.

Chapter III

Survival of early stripped eggs of the Noble crayfish *Astacus astascus* and effects of saline solution during artificial incubation

Seemann UB, Lorkowski K, Schiffer M, Hörterer C., Slater MJ, Buck BH



published in *Freshwater Crayfish* (2014)

DOI 10.5869/fc.2014.v20-1.1

Due to copyright policies of the publisher the article is linked via the above mentioned DOI.

Chapter IV

Polyculture potential of Red Nile tilapia (*Oreochromis niloticus*) and Noble crayfish (*Astacus astacus* Linnaeus, 1758) in a recirculating aquaculture system

Seemann UB, Kröncke N, Slater MJ, Buck BH



Full title

Polyculture potential of Red Nile tilapia (*Oreochromis niloticus*) and Noble crayfish (*Astacus astacus* Linnaeus, 1758) in a recirculating aquaculture system

Authors: Uli B. Seemann¹, Nina Kröncke^{1,2}, Matthew J. Slater¹, Bela H. Buck^{1,2}

Authors' institutions

¹ Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, D-27570 Bremerhaven, Germany

² University of Applied Sciences Bremerhaven, D-27568 Bremerhaven, Germany

Corresponding author

Uli B. Seemann, Bussestr. 27, 27570 Bremerhaven, phone 00 49 (0)471-4831-2813, fax 0049 (0)471-4831-1149, uli.seemann@awi.de

Running title

Polyculture of red Nile Tilapia and noble crayfish

Keywords

Crayfish, Cichlid, feed, integrated multi-trophic aquaculture

Abstract

The noble crayfish (*Astacus astacus*, Linnaeus) was held in two different kinds of polyculture with the finfish red Nile tilapia (*Oreochromis niloticus*) in a recirculating aquaculture system. The systems consisted of tanks for the fish and integrated containers for the crayfish. In the first treatment (T1) the tilapia were allowed to enter the crayfish containers, in the second treatment (T2) not. The stocking densities per m² were 25 (crayfish) and nine (tilapia). The impact of integrated stocking on growth and feed utilization of the two species was examined, as was the potential to minimize tank cleaning requirements. Controls of crayfish were held to allow comparisons of individual growth. Significantly higher growth rates were observed for both species in T1. Both, crayfish (ANOVA, $p > 0.05$; $f = 66.259$) and tilapia (tukey-test, $p < 0.05$, $f = 99.191$), achieved significantly higher growth rates in comparison to T2. However, crayfish exhibited similar growth rates in monoculture experiments which run parallel to T1 and T2 indicating that tilapia had a direct growth benefit while crayfish performed unaffected from tilapia presence/ absence. In conclusion, noble crayfish is suitable for polyculture with direct contact to finfish if appropriate species, densities and diets are selected. As an indicator species, red Nile tilapia successfully consumes crayfish biodeposits and show their potential for crayfish polyculture. In addition, the biodeposit consumption and tank turbation of tilapia negated any efforts of tank cleaning which is a problem in crayfish monoculture.

Introduction

Diet costs constitute more than 50% of the operating costs in intensive aquaculture (El-Sayed, 2006; Pillay & Kutty 2005). Therefore, effective and sustainable feed management is one of the most important tasks in aquaculture while scarcity of resources and increasing prices for raw materials are challenges, which demand novel approaches. Replacement of fish meal and fish oil with plant biomass or other components can reduce feeding costs (Izquierdo et al., 2005; Montero et al., 2003; Mourente & Bell, 2006). Additionally, the cultivation of different species in polyculture, also referred as integrated aquaculture, can significantly improve feed use efficiency and may reduce suspended particle loading and dilution in aquaculture systems (Piedrahita, 2003) by utilizing dissolved nutrients, recycling faeces and uneaten food into biomass. In polyculture, resources like energy, nutrients and water can be used efficiently and repeatedly (Lekang, 2008; Lutz, 2003). In addition, reduction in particle loading in recirculating aquaculture systems (RAS) can reduce stress on candidate species, tank cleaning frequency, manual labor costs and associated disturbance of culture animals.

To test this approach, the noble crayfish (*Astacus astacus*) and the red Nile tilapia (*Oreochromis niloticus*) were reared in a novel RAS polyculture to examine their potential of co-culture in terms of growth and feed utilization of both species.

The noble crayfish is a native, extremely high value species, which was once found in nearly all European freshwater systems (Franke, Wessels, & Horstgen-Schwark, 2011; Ingle, 1997; Taugbøl & Skurdal, 1988; Westman, 2002) and was a common food source in Europe (Cukerzis, 1988; Holdich, 2002; Skurdal & Taugbøl, 2001). Currently, crayfish are cultured in pond systems (Ackefors, 2000; Cukerzis, 1988), where the economic risk of a crayfish plague infection is high and the growth period is limited to a maximum of six months per year (Füreder, 2009; Taugbøl & Skurdal, 1988). The intensive work load and high mortality rates due to predation and cannibalism are also limiting factors for larger farms (Daws, Grills, Konzen, & Moore, 2002; Dethlefs, 2007; Hager, 2003; Usio, Konishi, & Nakano, 2001). As enclosed systems, RAS offer secure and adaptable culture conditions for reliable production rates, effectively free of disease concerns and unlimited by seasonal growth suppression (Lawson, 1995;

Seemann, Lorkowski, Slater, Buchholz, & Buck, 2014). Present polyculture experiments with crayfish are limited predominantly in connection with tropical species like the thermophile tilapia. In Mexico and the USA, for example, the Australian redclaw crayfish (*Cherax quadricarinatus*) and the American red swamp crayfish (*Procambarus clarkii*) were held together with Nile tilapia (*Oreochromis niloticus*) in ponds (Brummett & Alon, 1994; Martino & Wilson, 1986; Saad & Habashy, 2002). Polyculture with the European noble crayfish have been accomplished up to now only a few. Holm (1989) examined the polyculture of *A. astacus* with the Atlantic salmon (*Salmo salar*). A raised mortality was ascertained during the moulting phase of the crayfish, presumably on account of the higher stress level caused by the activities of the salmon. Moreover, a raised mortality could also be determined by the salmon in polyculture as well, although with a better growth of the fish at the same time. It was supposed that the higher growth of the fish is due to crayfish activity, because the stressed crayfish whirled up the sediment with the uneaten food and faeces, so that the suspended matter was available to the salmon as another food source (Holm, 1989).

The freshwater Nile tilapia originated in central and northern Africa and is now one of the most important finfish aquaculture species worldwide (El-Sayed 2006). The species is highly adaptable to varying culture conditions and a robust omnivore with correspondingly flexible dietary requirements (Suresh and Lin 1992). Tilapia are capable of filtering suspended particles above a size of about 100 µm, further expanding their available diet spectrum (El-Sayed, 2006; Klinkhardt, 2012). Different feeding attempts with camel and cowpat (Alhadhrhmi and Yousif, 1994) and pelleted outflow of cow dung biogas plant (GOPAL et al., 1996) show that tilapia is able to digest undigested components from the faeces of other species and to improve therefore the food utilization (Horvath and Tamas, 1984). Tilapia also distinguishes them by an easy handling, robustness and they are relatively modest toward water parameters (El-Sayed, 2006).

The aim of this project was the polyculture of both, Nile tilapia and the noble crayfish, which may reduce or render additional feeding of the fish, depending on stocking rate and overall feed ratio. The faeces and uneaten diet from the crayfish may be effectively converted to fish

biomass, increasing the economic efficiency of the system. In addition, cleaning costs especially of the tank bottom due to finfish activity may be minimized or eliminated. Despite a clear potential of this co-culture very few trials have been carried out in the polyculture of crayfish and finfish. Further, the socialization of these both species has not been examined fully enough to be able to make distinct statements in relation to species interactions, systems design and/or culture and utilization concepts. The current study documents and compares the growth rates of *A. astacus* and *O. niloticus* in polyculture and monoculture and examines the influence of crayfish faeces and uneaten crayfish diet on the growth performance of tilapia. The effects of polyculture on tank cleaning rates are also investigated.

Materials and Methods

Experimental animals

Crayfish was obtained from a commercial pond system at a crayfish farm (Trout and crayfish farm Göckemeyer, Poggenhagen, Germany), transferred to the Centre of Aquaculture Research (AWI, Bremerhaven, Germany), and maintained in RAS devices. The crayfish was about two years old at the beginning of this study. All male tilapia were received from a tilapia farm (Kirschauer Aquakulturen, Kirschau, Germany), and about five weeks old prior to the start of the experiment.

Experimental design

Polyculture treatments

Two polyculture treatments were established sequentially:

- Treatment 1 (T1): tilapia was free to enter appropriate perforated crayfish containers (Transoplast, Emmerich am Rhein, Germany) and feed on excess feed and faeces over 84 days. Crayfish feed was given on feeding plates and then passed through perforated container bottom to the tank bottom by crayfish movement.

- Treatment 2 (T2): tilapia had no direct contact with the crayfish due to perforated but meshed (mesh size: 6 mm) crayfish containers where tilapia could not pass through (Fig. 4). The feeding and experiment time was similar to feeding in treatment A.
- The crayfish in each treatment were subdivided into two feeding group C1 and C2. C1 was fed with a single carp while C2 was fed with a diet mix containing higher lipid and protein amounts.

The crayfish was stocked in six rotary stacking containers (0.6 x 0.4 cm), which were part of a freshwater recirculating aquaculture system (RAS) consisting of three culture tanks (125 x 90 x 125 cm) with two build-in containers each and external water treatment devices. The process water drained through pipes at the bottom of the tanks, which were connected with a mechanical filter and a biofilter. The normal distributed crayfish ($n = 36$, $p > 0.05$, ANOVA) was randomly dispensed to the six containers with stocking densities 25 animals per m^2 . One replicate of each group was placed into one tank. The sex ratio was 1:1. Treatment 1: Start weight was 13.9 ± 6.0 g at 3.7 ± 0.6 cm carapace length (group C1) and 14.0 ± 8.5 g at 3.8 ± 0.7 cm carapace length (group C2). Treatment 2: Start weight was 14.7 ± 0.1 g at 3.7 ± 0.1 cm carapace length for both groups. The crayfish was marked with numbered and colour-coded plastic carapace labels to monitor individual growth and moulting (Bienencenter, Kronau, Germany). Crayfish total body weight (accuracy ± 0.1 g), carapace length (accuracy ± 0.1 mm) and sex were recorded every three weeks. The moults were documented every day. Each container was fitted with 21 plastic tubes (4 cm inner diameter and 10 cm length), which acted as shelters for the crayfish. All tanks were checked daily for moulting and mortalities.

Normal distributed tilapia ($n = 36$, $p > 0.05$, ANOVA) were stocked in three culture tanks at a density of nine animals per m^3 . Start weight was 1.2 ± 0.1 g at 4.1 ± 0.1 cm for treatment 1 and 5.7 ± 0.02 g at 7.0 ± 0.1 cm for treatment 2. Data on total body weight (accuracy ± 0.1 g) and total body length (accuracy ± 0.1 mm) was collected every two weeks. The tanks and species were examined daily for malfunctions and mortalities.

In addition, normal distributed crayfish ($n = 6$, $p > 0.05$, ANOVA) was held individually in six glass aquaria (25 x 16 x 15.5 cm) in two groups ($n = 3$) parallel to treatment 1 and 2. Aquaria were equipped with plastic tubes of the same size mentioned above and with an internal filter (IN 300 Plus, Tetra, Melle, Germany). Feeding and measurements were similar to polyculture experiments to allow comparisons of growth rate under individual and polyculture conditions (Individual housing 1 (IH1) + T1; Individual housing 2 (IH2) + T2)

Feeding regime

Crayfish was fed with extruded sinking pellets. Group C1 was fed with a carp feed (Aller Classic, Aller Aqua Emsland, Germany), 2 mm in diameter, containing 30 % protein and 7 % fat. The second group ("Astax + K2") were fed with a mixture of a trout feed to 80 % (Astax 44/22, Muskator Werke, Düsseldorf, Germany), 5 mm in diameter, containing 44 % protein and 22 % fat and another carp feed to 20 % (Cyprinin K2, Muskator Werke, Düsseldorf, Germany), 2 mm in diameter, which contains 25 % protein and 6.8 % fat. The feed ratio was 2.5% of crayfish body mass and fed every two days at 5:00 pm after Seemann et al. (2014). Tilapia was unfed and therefore dependent on uneaten diet remains of the diets and faeces of the crayfish. In T2, feed and faeces were removed before the next feeding and transferred into the tank for tilapia consumption.

Water parameters

The water parameters were set as followed: pH 8.0, general hardness 13 dGH, carbonate hardness 6 dKH and O₂ content 105.2%. The concentrations of the nutrients were as followed: ammonium 0.02 mg L⁻¹, nitrite 0.013 mg L⁻¹ and nitrate 30.7 mg L⁻¹. Temperature in all tanks/aquaria was adjusted to 20°C. Water parameters were measured weekly using a digital meter (WTW Multi 3430, Weilheim, Germany). Further, ammonium (NH₄⁺), nitrate (NO₃⁻) and nitrite (NO₂⁻) were measured weekly using a VIS-Spectral photometer (DR 2800, Hach Lange, Düsseldorf, Germany) (accuracy ± 0.1 nm) and reagent kits for photometric analysis

(Hach Lange, Düsseldorf, Germany). The general hardness and carbonate hardness were quantified using a test kit for freshwater (Tetra GmbH, Melle, Germany).

Statistical analyses

The growth rates of crayfish and tilapia were calculated as the specific growth rate (SGR). The feed conversion ratio (FCR) was calculated with the dry weight of consumed feed and the wet gain weight to indicate the suitability of the used feeds and to draw comparisons to other studies. The body weight (BW) and the body length (BL) were used to calculate the condition index (CI) for a general indication of the health status of tilapia. The mortality rate ($M\%$) was calculated with the initial number of specimens per unit N_{initial} and the number of specimen at the end N_{final} .

$$\text{SGR} = 100 \times (\ln \text{BW}_{\text{final}} (\text{g}) - \ln (\text{BW}_{\text{initial}} (\text{g})) / \text{time} (\text{d}) \quad (1)$$

$$\text{FCR} = \text{dry weight of feed consumed (kg)} / \text{wet weight of gain (kg)} \quad (2)$$

$$\text{CI} = \text{BW (g)} / \text{BL}^3 (\text{cm}) \quad (3)$$

$$\text{M} = 100 \times (1 - N_{\text{final}}/N_{\text{initial}}) \quad (4)$$

Statistical analyses were carried out using SigmaPlot version 12.5 (Build 12.5.0.38, Systat Software Incorporation). All data were tested with a Shapiro-Wilk test for normality distribution and with a Bartlett's test for the homogeneity of variance. Growth rates were compared between treatments using a one-way ANOVA, followed by the *post-hoc* Tukey test for multiple comparisons of means. A 95% confidence level was presumed.

Results

Growth

Tilapia: in T1 four animals passed away, which led to an average mortality rate of 11.1%. Some fish showed malfunctions and abnormalities, such as a burst bile, green liver color

and fat depositions in the abdominal cavity but overall the amount of twelve crayfish including their produced faeces per tank and remaining feed resulted in considerable tilapia growth without any additional feeding of the tilapia. Biomass increased by 178.8 % and 38.0 % in length and the final condition index remained at 1.7 g cm⁻³. In T2 biomass increased by 57.0 % and 16.6 % in length, the final condition index remained at 1.5 g cm⁻³. While no mortality occurred in T2, the tilapia in T1 reached significantly higher growth rates (tukey-test, $p < 0.05$, $f = 99.191$).

Table 1: Overall tilapia growth in T1 and T2 at 20°C, mean ± SD (n = 3).

Group	Mean weight		Mean length		CI		SGR _B [% BW d ⁻¹]
	[g]		[cm]		[g cm ³]		
	Initial	Final	Initial	Final	Initial	Final	
Treatment 1	1.17	3.27	4.08	5.66	1.7	1.7	1.22
	± 0.41	± 1.96	± 0.50	± 1.27	± 0.06	± 0.17	
Treatment 2	5.72	8.98	6.99	8.15	1.5	1.5	0.54
	± 2.54	± 5.57	± 1.19	± 1.76	± 0.06	± 0.00	

Crayfish: the mortality over the entire experimental period was 16.7%. Mortality resulted from cannibalism during exuviation or failed exuviation. Because of no significant differences between group C1 and C2, these data sets were summarized (n = 6) for further analysis. In T1, biomass increased by 24.9% and 9.1% in length. In T2 a biomass increase of 3.1% was measured while length increment was 0.2 %. Final weight (ANOVA, $p > 0.05$; $f = 22.951$), length (ANOVA, $p > 0.05$; $f = 60.860$) and specific growth rate (ANOVA, $p > 0.05$; $f = 66.259$) of the crayfish in the T1 differ significantly from the final weight, length and specific growth rate in T2. A total of 39 moults were documented over the experimental period of 84 days in T1. Apart from five animals, every crayfish moulted at least once, maximum twice during this experiment. In T2 only two crayfish moulted.

Interactions: no aggression or exclusion was monitored in species interactions at any stage during the experimental period. In both trials, feed and faeces were completely consumed by tilapia. Besides, the feeding of the feed Astax 44/22 caused a considerable fat film on the water surface, which entirely disappeared by turbation of the tilapia after a few hours.

Table 2: Crayfish parameters for T1 and T2, mean \pm SD (n = 6).

Group	Mean weight		Mean length		SGR [% BW d ⁻¹]
	[g]		[cm]		
	Initial	Final	Initial	Final	
Treatment 1	13.91	17.38	3.68	4.02	0.27
	± 6.30	± 7.62	± 0.59	± 0.58	
Treatment 2	14.90	15.37	3.73	3.74	0.07
	± 7.59	± 7.77	± 0.67	± 0.66	

Individual housing (IH)

In IH1, biomass of crayfish increased by 38.5 % and 12.7 % in length. Eight crayfish moulted during trial period in IH1, which ran parallel to T1. In IH2, biomass increased by 1.4 % and 1.9 % in length while no moulting occurred, simultaneously to T2. No crayfish died over the experimental periods.

Table 3: Crayfish parameters for the individual housing experiment, mean \pm SD (n = 6).

Group	Mean weight		Mean length		SGR [% BW d ⁻¹]
	[g]		[cm]		
	Initial	Final	Initial	Final	
Individual housing (I)	12.40	17.19	3.67	4.13	0.37
	± 1.02	± 3.44	± 0.15	± 0.30	
Individual housing (II)	11.98	12.15	3.53	3.60	0.02
	± 0.11	± 0.26	± 0.05	± 0.08	

Crayfish growth rates of the individual held crayfish (IH) and grouped crayfish (T) were similar to each other. The animals in the polyculture trials reached no significantly higher growth rates (ANOVA, $p > 0.05$, $f = 2.015$) than the monocultured animals.

All water parameters ranged as followed: temperatures as adjusted: $20.6 \pm 0.1^\circ\text{C}$, pH: 7.4 - 8.0, general hardness: 11 - 13 dGH, carbonate hardness: 6 - 8 dKH, O_2 : 102 - 107%, ammonium: 0.01 - 0.04 mg L⁻¹, nitrite: 0.01 - 0.26 mg L⁻¹ and nitrate: 5.2 - 30.7 mg L⁻¹.

Discussion

Crayfish performance

Mesasured growth of noble crayfish is comparable with viable natural and commercial growth rates and corresponds to two year old crayfish indicating the general suitability of the used crayfish feeds and amounts (Hager, 2003).

Although especially in T1 the tilapia could pass unimpeded into the rotary stacking container, may have challenged with the noble crayfish for the feed and may disturbed them, growth rates of crayfish were not negatively influenced. The crayfish reached comparable growth rates in polyculture individual housing experiments. Therefore, it can be assumed that tilapia do not constitute a stress factor for the crayfish at the used densities. Also other studies on polyculture of tilapia with e.g. Australian red claw crayfish (*Cherax quadricarinatus*) and American red swamp crayfish (*Procambarus clarkii*) showed that the presence of tilapia had no negative effects on the growth of the crayfishes (Brummett & Alon, 1994; Martino & Wilson, 1986; Saad & Habashy, 2002). The effect of higher densities remains unclear.

At an age of two years crayfish moult from four to five times and in their third year from two to three moults per year (Cukerzis, 1988; Hager, 2003). With increasing age and size, the number of moulting events decreases (Taugbøl & Skurdal, 1992). Due to the experiment duration of three months it can be assumed that animals moulted prior to T_0 and after T_1 .

The reasons for the mortality of noble crayfish can be explained by cannibalism of conspecifics during or shortly after the moulting process. The aggressiveness and the inclination to cannibalism rises with increasing age from which a decreased growth and a lower survival

rate results (Franke et al., 2011). The presence of Tilapia in the direct polyculture did not result in crayfish mortality.

Tilapia performance

Consideration of the growth rates clearly indicate that under the given conditions, feeding of the tilapia exclusively with faeces and uneaten feed of crayfish is possible. It could not be unequivocally proved that fish faeces contributed directly to the nutrition of the tilapia, because the faeces of the crayfish are not stable for long and dissolve relatively fast in the water (Janphiro, Chaiprasert, Thongthieng, Suwanthep, & Songkasiri, 2010). So the primary nutrition of the fish in polyculture based presumably on the uneaten feed of the crayfish. On this basis, it cannot be excluded that this kind of feed is an equivalent substitute for the tilapia.

Nile tilapia need a balanced nutrition, which provides them enough proteins with essential amino acids, carbohydrates, fats, vitamins, minerals and dietary fiber. Juvenile fish have a higher protein need between 30% to 56% (Hafedh, 1999; Jauncey, 1982; Siddiqui, Howlander, & Adam, 1988). Therefore, the used crayfish feed with a protein content of 30% could have been too low for the juvenile fish and caused a poor growth increment than stated in literature (El-Sayed 2006). The lipid content should be located for tilapia with a size of 2.5 g to 7.5 g between 4.4% and 5.2%. To maximize the protein utilization, a lipid content of 8% to 12% is recommended for tilapia with a size up to 25 g (Chou & Shiau, 1996; Jauncey, 1982).

It is supposed that the digestive problems of the tilapia arise from the interactions of different factors like an unbalanced diet, indigestible feed components and a low water temperature, causing the metabolic disorder and a weak immune system of the fish.

These affections often appear when animals are held longer time under suboptimal conditions, especially at low temperatures (< 20°C) (Klinkhardt, 2012; Le Morvan, Troutaud, & Deschaux, 1998). Therefore, it is possible that the low temperature of 20°C had an essential role in causing food problems as temperature is one of the most important factors concerning the metabolism of the tilapia (El-Sayed, 2006). The temperature optimum of tilapia is located at 28°C which favors a quick growth and a good feed utilization (Balarin & Haller, 1982;

Chervinski, 1982; Klinkhardt, 2012; Philippart & Ruwet, 1982). Besides, the enzyme activity of the tilapia sinks with decreasing temperature which can cause lower or worse insufficient feed component digestion.

Conclusion

The general potential of tilapia concerning the utilization of incurred solids, in form of uneaten feed and faeces, is high. Tilapia is able to minimize the incurred solids of the noble crayfish and proved any additional cleaning of the tanks redundant in the polyculture experiments. Thereby, the effective utilization of solids from noble crayfish was raised.

The presence of the tilapia did not reduce the growth of the crayfish and an integrated culture of both species in a system proved to be possible. The used densities suggest a system lower water level and higher densities of crayfish than tilapia, were finfish are primary used as a tank cleaner und biodeposit converter. Other suitable candidates for socialization with the tilapia are possibly shrimps or other crayfish species, which show the same demands for the culture parameters as the fish, especially regarding temperature regime. The polyculture of *O. niloticus* with *Cherax quadricarinatus* and *Procambarus clarkii* indicated in different polyculture studies the first possible potentials of an integrated aquaculture of both species. But these studies are limited to pond culture, so further studies should examine this issue in RAS.

Noble crayfish are suited for a polyculture with other species as well. *A. Astacus* lives on the ground and as an omnivore is able to feed on sedimented fish faeces and uneaten food. Suitable candidates for other polyculture studies with crayfish are possibly salmon trout or hybrid striped bass, because the culture parameters of these species are similar. It is obvious on this occasion, to combine or integrate furthermore omnivorous species which are able to convert the suspended solids in the low trophic level in biomass. If the species are combined not only exclusively under the aspect of the solid reduction in the system, but also in regard to a production of food fish, then species should be combined which are more similar to themselves in the culture parameters and nutritional requirements.

Acknowledgement

The authors would like to thank Marcus Thon and Kai Lorkowski for their technical assistance and for the auxiliary readiness with the procurement of the tilapia. We are also grateful to Jörn Halfer, Sönke Gast, Gregor Jähne and Christina Hörterer for their assistance and team work.

References

- Ackefors, H. E. G. (2000). Freshwater crayfish farming technology in the 1990s: a European and global perspective. *Fish and Fisheries*, 1(4), 337-359. doi:10.1046/j.1467-2979.2000.00023.x
- Balarin, J. D., & Haller, R. D. (1982). The intensive culture of tilapia in tanks, raceways and cages. In J. F. Muir & R. J. Roberts (Eds.), *Recent Advances in Aquaculture* (pp. 267-355). London, Canberra, Boulder Colorado: klinkCroom Helm, Westview Press.
- Brummett, R. E., & Alon, N. C. (1994). Polyculture of Nile tilapia (*Oreochromis niloticus*) and Australian red claw crayfish (*Cherax quadricarinatus*) in earthen ponds. *Aquaculture*, 122(1), 47-54. doi:10.1016/0044-8486(94)90332-8
- Chervinski, J. (1982). *Environmental physiology of tilapias*. Paper presented at the ICLARM Conference, Manila, Philippines.
- Chou, B. S., & Shiau, S.-Y. (1996). Optimal dietary lipid level for growth of juvenile hybrid tilapia, *Oreochromis niloticus* x *Oreochromis aureus*. *Aquaculture*, 143, 185 – 195.
- Cukerzis, J. M. (1988). *Astacus astacus* in Europe. *Freshwater Crayfish, Biology (Management and Exploitation)*.
- Daws, A. G., Grills, J., Konzen, K., & Moore, P. A. (2002). Previous Experiences Alter the Outcome of Aggressive Interactions Between Males in the Crayfish, *Procambarus Clarkii*. *Marine and Freshwater Behaviour and Physiology*, 35(3), 139-148. doi:10.1080/1023624021000014725
- Dethlefs, A. (2007). *Predator - Prey - Interaction between Dragonfly Larva (Odonata: Anisoptera) and Crayfish Juveniles (Astacus astacus)*. (Diploma Diplomatehsis), Christian - Albrechts - University Kiel, Kiel, Germany.
- El-Sayed, A.-F. M. (2006). *Tilapia culture*. Oxfordshire (UK), Cambridge (USA): CABI Publishing, CAB International.
- Franke, R., Wessels, S., & Horstgen-Schwark, G. (2011). Enhancement of survival and growth in crowded groups: the road towards an intensive production of the noble crayfish *Astacus astacus* L. in indoor recirculation systems. *Aquaculture Research*, 44(3), 451-461. doi:DOI 10.1111/j.1365-2109.2011.03052.x

- Füreder, L. (2009). *Flusskrebse Biologie - Ökologie - Gefährdung* (Vol. 6). Wien: Folio Verlag.
- Hafedh, Y. S. A. (1999). Effects of dietary protein on growth and body composition of Nile tilapia, *Oreochromis niloticus* L. *Aquaculture Research*, 30(5), 385-393. doi:10.1046/j.1365-2109.1999.00343.x
- Hager, J. (2003). *Edelkrebse - Biologie, Zucht, Bewirtschaftung* (2., überarbeitet Auflage ed.). Graz - Stuttgart: Leopold Stocker Verlag.
- Holdich, D. M. (2002). Distribution of crayfish in Europe and some adjoining countries. *Bulletin Français de la Pêche et de la Pisciculture*(367), 611-650. doi:10.1051/kmae:2002055
- Holm, J. C. (1989). Atlantic Salmon Parr (*Salmo salar*) and Noble Crayfish (*Astacus astacus*) in Duoculture. *Aquacultural Engineering*, 8(2), 79-94. doi:Doi 10.1016/0144-8609(89)90006-X
- Ingle, R. (1997). *Crayfishes, lobsters and crabs of Europe - An illustrated guide to common and traded species* (Vol. XI): Springer.
- Izquierdo, M. S., Montero, D., Robaina, L., Caballero, M. J., Rosenlund, G., & Ginés, R. (2005). Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. *Aquaculture*, 250(1–2), 431-444. doi:http://dx.doi.org/10.1016/j.aquaculture.2004.12.001
- Janphiro, T., Chairprasert, P., Thongthieng, T., Suwanthep, S., & Songkasiri, W. (2010). Increasing fish feed stability using guar gum: case study with *Channa striata*. *Journal of Agricultural & Food Industrial Organization*, 3, 363-370.
- Jauncey, A. (1982). The effect of varying dietary protein level on growth, food conversion, protein utilization and body composition of juvenile tilapias (*Sarotherodon mossambicus*). *Aquaculture*, 27, 43 – 54.
- Klinkhardt, M. (2012). *Niltilapia – Maßgeschneidert für die Aquakultur*. Hamburg: Fachpresse Verlag.
- Lawson, T. (1995). Recirculating Aquaculture Systems *Fundamentals of Aquacultural Engineering* (pp. 192-247): Springer US.
- Le Morvan, C., Troutaud, D., & Deschaux, P. (1998). Differential effects of temperature on specific and nonspecific immune defences in fish. *The Journal of Experimental Biology*, 201, 165-168.
- Lekang, O.-I. (2008). *Aquaculture Engineering*: Wiley-Blackwell.
- Lutz, C. G. (2003). Principles of polyculture. *Aquaculture Magazine*, 29(2), 34-39.
- Martino, C., & Wilson, J. L. (1986). Behavioral interactions of tilapia, crawfish and freshwater prawns in aquaculture. *Aquacult. Mag.*, 12(4), 36-37.
- Montero, D., Kalinowski, T., Obach, A., Robaina, L., Tort, L., Caballero, M. J., & Izquierdo, M. S. (2003). Vegetable lipid sources for gilthead seabream (*Sparus aurata*): effects on

- fish health. *Aquaculture*, 225(1–4), 353-370. doi:[http://dx.doi.org/10.1016/S0044-8486\(03\)00301-6](http://dx.doi.org/10.1016/S0044-8486(03)00301-6)
- Mourente, G., & Bell, J. G. (2006). Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (*Dicentrarchus labrax* L.) over a long term growth study: effects on muscle and liver fatty acid composition and effectiveness of a fish oil finishing diet. *Comp Biochem Physiol B Biochem Mol Biol*, 145(3-4), 389-399. doi:10.1016/j.cbpb.2006.08.012
- Philippart, J.-C. L., & Ruwet, J.-C. L. (1982). *Ecology and distribution of tilapias*. Paper presented at the ICLARM Conference, Manila, Philippines.
- Piedrahita, R. H. (2003). Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture*, 226(1-4), 35-44. doi:10.1016/s0044-8486(03)00465-4
- Pillay, T. V. R., & Kutty, M. N. (2005). *Aquaculture: Principles and Practices* (Vol. 2nd Edition): Wiley-Blackwell.
- Saad, A. S., & Habashy, M. M. (2002). Survival and growth rates of early stages of some fish species reared with the freshwater crayfish, *Procambarus Clarkii* (Girard, 1852). *Egypt. J. Aquat. Biol. / Fish.*, 6(3), 163-180.
- Seemann, U. B., Lorkowski, K., Slater, M. J., Buchholz, F., & Buck, B. H. (2014). Growth performance of Noble crayfish *Astacus astacus* in recirculating aquaculture systems. *Aquaculture International*, 23(4), 997-1012. doi:10.1007/s10499-014-9859-2
- Siddiqui, A. Q., Howlander, M. S., & Adam, A. A. (1988). Effects of dietary protein levels on growth, diet conversion and protein utilization in fry and young Nile tilapia, *Oreochromis niloticus*. *Aquaculture*, 70, 63 – 70.
- Skurdal, J., & Taugbøl, T. (2001). Crayfish of commercial importance. In D. M. Holdich (Ed.), *Biology of Freshwater Crayfish* (pp. 467-510). Oxford: Blackwell Science.
- Taugbøl, T., & Skurdal, J. (1988). Increased proportion of mature females of the Noble Crayfish (*Astacus astacus* L.) in culture conditions. *Aquaculture*, 69(1-2), 39-42. doi:[http://dx.doi.org/10.1016/0044-8486\(88\)90184-6](http://dx.doi.org/10.1016/0044-8486(88)90184-6)
- Taugbøl, T., & Skurdal, J. (1992). Growth, mortality and moulting rate of noble crayfish *Astacus astacus* L., juveniles in aquaculture experiments. *Aquaculture and Fisheries Management*, 23, 411-420.
- Usio, N., Konishi, M., & Nakano, S. (2001). Species Displacement Between an Introduced and A 'vulnerable' Crayfish: The Role of Aggressive Interactions and Shelter Competition. *Biological Invasions*, 3(2), 179-185. doi:10.1023/A:1014573915464
- Westman, K. (2002). Alien crayfish in Europe: negative and positive impacts and interactions with native crayfish. In E. Leppäkoski, S. Gollasch, & S. Olenin (Eds.), *Invasive Aquatic*

Appendix

Table 10: Manufacturer's data for the carp feed Cyprinin K2 (Muskator Werke, Düsseldorf, Germany). Further ingredients of the carp feed Cyprinin K2 (Muskator Werke, Düsseldorf, Germany) are soya extraction grist (steam-heated), wheat bran, wheat, linseed extraction grist, maize, post-extraction rapeseed meal, malt sprouts, wheat flour, vegetable fat, malt spent grains (dried), barm (dried), sunflower seed, calcium carbonate and sodium chloride.

Analytical components	%	Trace elements	mg kg⁻¹
Crude protein	25.0	E1 Iron (Iron(II)carbonate)	90.0
Crude fat	6.8	E2 Iodine (Calcium iodate)	0.3
Raw fiber	6.4	E3 Cobalt (Cobalt sulfate, Heptahydrate)	0.3
Raw ash	6.9	E4 Copper (Copper sulfate, Pentahydrate)	15.0
Sodium concentration	0.2	E5 Manganese (Manganese oxide)	75.0
Calcium concentration	1.0	E6 Zinc (Zinc oxide)	90.0
Phosphorus concentration	0.7	E8 Selenium (Sodium selenite)	0.4
Vitamins	IE kg⁻¹		
	1		
Vitamin A (E672)	27000		
Vitamin C	2200		
Vitamin D3 (E671)	2500		
Vitamin E	200		
Grain size [mm]		2.0	

Table 11: Manufacturer's data for the carp feed Aller Classic (Aller Aqua, Emsland, Germany). Further ingredients of the carp feed Aller Classic (Aller Aqua, Emsland, Germany) are triticale, wheat, distillers dried grains with solubles (DDGS), rape seed, haemoglobin meal, sunflower seed, fish meal, hydrolysed protein and rape seed oil. Information about existing trace elements or antioxidants are not specified by the producer.

Analytical components	%
Crude protein	30.0
Crude fat	7.0
Raw fiber	4.5
Raw ash	5.9
Nitrogen concentration	5.4
Phosphorus concentration	1.1
Vitamins	IE kg⁻¹
Vitamin A (E672)	10000
Vitamin D3 (E671)	1000
Vitamin E	200
Grain size [mm]	2.0

Table 12: Manufacturer's data for the trout feed Astax 44/20 (Muskator Werke, Düsseldorf, Germany). Further ingredients of the trout feed Astax 44/22 (Muskator Werke, Düsseldorf, Germany) are fish meal, fish oil, soya extraction grist, wheat, pea protein, haemoglobin powder and rape seed.

Analytical components	%	Trace elements	mg kg⁻¹
Crude protein	44.0	E1 Iron (Iron(II)carbonate)	100
Crude fat	22.0	E2 Iodine (Calcium iodate)	1.6
Raw fiber	2.0	E3 Cobalt (Cobalt sulfate, Heptahydrate)	0.8
Raw ash	6.0	E4 Copper (Copper sulfate, Pentahydrate)	4.8

Calcium concentration	1.0	E5 Manganese (Manganese oxide)	16
Phosphorus concentration	10	E6 Zinc (Zinc oxide)	24
		E8 Selenium (Sodium selenite)	0.08
Vitamins	IE kg⁻¹	Antioxidants	mg kg⁻¹
Vitamin A (E672)	12000	E321 Butyl hydroxytoluene (BHT)	120
Vitamin D3 (E671)	1600		
Vitamin E	160		
Dye stuffs	mg kg⁻¹		
	1		
Astaxanthin (E161j)	55.0		
Cantaxanthin (E161g)	25.0		
Grain size [mm]		5.0	

Chapter V

Shelter preference of Noble crayfish *Astacus astacus* in recirculating aquaculture systems

Seemann UB, Hörterer C, Buck BH



Manuscript

Title

Shelter preference of Noble crayfish *Astacus astacus* in recirculating aquaculture systems

Authors

Uli B. Seemann¹, Christina Hörterer¹, Bela H. Buck^{1,2}

Authors' institutions

¹ Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, D-27570 Bremerhaven, Germany

² University of Applied Sciences Bremerhaven, D-27568 Bremerhaven, Germany

Corresponding author

Uli B. Seemann, Bussestr. 27, 27570 Bremerhaven, phone 00 49 (0)471-4831-2813, fax 0049 (0)471-4831-2210, uli.seemann@awi.de

Keywords

shelter type, tubes, block-filter, wool-filter, Noble crayfish, RAS

Abstract

In the current study, experiments were carried out to test preferences of the Noble crayfish *Astacus astacus* for different shelter types. The suitability of different structures for crayfish size classes was measured to improve size-specific maintenance conditions in RAS. The experiment was conducted over a period of 20 days in a recirculation aquaculture system. Three different artificial shelter types were chosen by complexity of structure, building effort and their availability on the market to meet crayfish and RAS requirements. The numbers of the crayfish per shelter and outside were recorded daily in the morning over four weeks. In order to determine personal preference for shelter types each crayfish was marked individually. The three tested materials proved to be suitable for RAS since they are light, easy to handle and can be proceeded with moderate costs in considerable amounts. *Astacus astacus* showed a clear shelter preference. The highest percentage of individuals preferred tubes as shelter. A significant lower number of individuals preferred wool-filter and block-filter. Individual size class had a strong effect on shelter preference. While only few animals of size class 1 preferred tubes, a significant higher number of size class 2 and size class 3 used tubes. Size class also had a significant effect on the use of wool-filter. Wool-filter was preferred of size class 1 animals, while only small amounts of size class 2 and 3 were regularly found in this shelter type. Almost equal numbers of size class 1 and 2 individuals preferred block-filter. Around 41% of all individuals showed an individual shelter preference. Survival rate of crayfish was not affected by shelter type but shelter types should be altered in dependence of the crayfish age and length to reduce repression and growth variance between individuals.

Introduction

Crayfish harvest in Western Europe mainly based on candidates, such as *Astacus astacus* and *Astacus pallipes*, before the onset of crayfish plague and the decline of the native stock started (Harlioğlu, 2007; Holdich, 1993; Zimmermann, 2012). To date, *Astacus astacus* is the main endemic species of freshwater crayfish cultured to any extent in Germany. From an economic perspective this species is a relevant candidate for aquaculture as it fetches a high market price (Keller, 2017; Taugbøl & Skurdal, 1992). However, due to the fact that almost all of the noble crayfish harvest comes from ponds, crayfish plague introduced by alien crayfish species represent a threat to these pond-based aquaculture farms (Hager, 2003; Holdich, 1993). Beside diseases, competition and predation by other species within a pond causes juvenile loss. However, this disadvantage can be limited by tank rearing allowing controlled conditions (Franke, Wessels, & Horstgen-Schwark, 2011; Holdich & Lowery, 1988). Furthermore, growth, survival and production rates as well as feeding rates can be calculated when animals are reared in tanks.

Sustainable crayfish aquaculture can be achieved by using recirculating aquaculture systems (RAS), which offer several advantages, such as reduced water consumption, nutrient recycling, better hygiene/disease management as well as biological pollution control (Review by Martins et al., 2010). Yet, the complexity of the natural habitat is not met by those aquaculture systems. In nature *A. astacus* can be found in creeks and rivers with steep-sided banks providing shelter beneath roots or stones, while on loamy ground it inhabits burrows (Pöckl, 1998). Under natural conditions shelter is an important resource for crayfish as it reduces predation risk and reduces competition (Söderbäck, 1994). In aquaculture systems cannibalism is a major issue in crayfish culture (Celada et al., 1993; Gydemo & Westin, 1989; Skurdal & Taugbøll, 1994) and can be reduced by the use of adequate shelter (Jones, 1995; Jones & Ruscoe, 2001; Sáez-Royuela, Carral, Celada, & Pérez, 2001). Open pond farms use brick and sand stones as hiding structures, which go along with several disadvantages regarding crayfish handling and handling of the stones itself, especially when used in RAS. Different shelter types had a strong positive effect on survival of redclaw crayfish *Cherax quadricarinatus* probably due to mitigation of molt-related cannibalism by providing suitable shelter (Jones & Ruscoe, 2001). First results indicate that the use of fibre-cement sheets as shelter resulted in a higher survival rate than obtained with PVC pipes for stage-2 juvenile white-clawed crayfish *Austropotamobius pallipes* (Sáez-Royuela et al., 2001).

Fibre-cement are commonly used in crayfish farms as they can be inhabited by animals over a wide range of size classes (Kozák, Füreder, Kouba, Reynolds, & Souty-Grosset, 2011; Mitchell, Collins, & Austin, 1994). Shelter use, shelter acquisition and shelter eviction of crayfish depend, among other characteristics, on dominance and size class (Martin & Moore, 2008; Rabeni, 1985). Dominance is related to body size, while shelter use by one size class can be altered when larger animals are present (Rabeni, 1985).

In the current study, experiments were carried out to test preferences of the Noble crayfish *Astacus astacus* for different shelter types. We evaluated the effectiveness of three different structures for three crayfish size classes, which can improve size-specific maintenance conditions in RAS.

Material and Methods

Experimental design and setup

The experiment was conducted over a period of 20 sampling days in a black PE tank (1.44 m³) in a recirculation aquaculture system (RAS) at the Centre for Aquaculture Research (ZAF) of the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI), Germany, in July 2014.

The tank was divided into four compartments with an area of 100 x 50 cm each with one inflow per compartment. All single compartments were separated by a net (mesh size 6 mm) to

ensure constant flow-through. The separation wall was provided with a PVC stripe at the top to prevent crayfish escape. Water parameters (dissolved oxygen [%], pH and temperature [°C]) were measured on a daily basis. Total hardness (°dH), carbonate hardness (°dH), nitrate, nitrite and ammonium concentrations (mg/l) were measured weekly. The carbonate hardness was maintained at a constant level by adding calcium carbonate.

To test hiding preferences, three different artificial shelter types (tubes, block-filter, and wool-filter) were chosen by complexity of structure, building effort and their availability on the market. As shelters for RAS need to fulfill several characteristics like light weight, easy handling, low material and processing costs industrial polyvinylchlorid (PVC) tubes were selected for the following experiments. Pre-tests indicated that

crayfish to not hinder each other when direct compartments were selected which rendered spacer between these compartments. The tube shelter battery was built with 35 PVC-tubes (Ø 25 mm) in three levels with decreasing tube depths to the top (60, 80 and 100 mm) (Fig. 1A). One end of each tube was closed with a PVC stripe. This filter type proved to an easy to handle and good choice in previous studies regarding survival rates (Seemann, Lorkowski, Schiffer, et al., 2014; Seemann, Lorkowski, Slater, Buchholz, & Buck, 2014). The 2nd shelter was built from an AK-block filter (approx. 35 pipes, approx. Ø 25 mm, polypropylene; GEA 2H Water Technologies GmbH, Huerth, Germany) in three levels with decreasing depth (60, 80 and 100 mm) providing shelter for approximately 40 crayfish (Fig. 1B). The back end and the sides were closed with PVC-strips. The proportion of shelter : crayfish of these two filter types was approx. 1 : 3 to exclude neighbor effects from occupied, adjoined shelters.

The 3rd shelter (wool-filter) was built with a box of PVC-strips with the front and top open and plastic filter wool attached to the bottom stripe so that the filter wool did exceed eight cm height and a density of 8 g/l (Fig. 1C).

All shelter types had the same area and volume (300 mm x 100 mm x 80 mm). Each type was provided once in each compartment. The shelters were arranged alongside each other in one third of the compartment leaving enough space in the front and back for the foraging activities

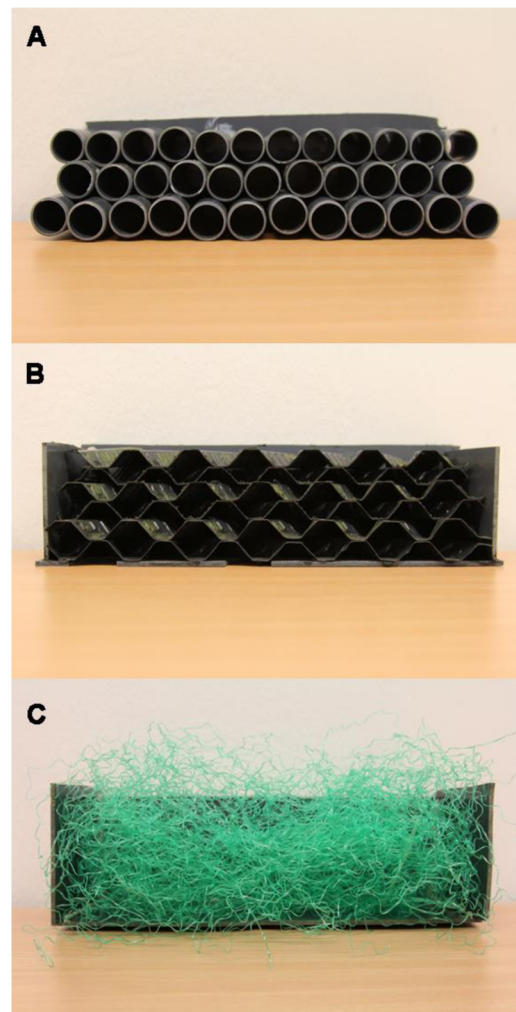


Figure 1 Shelter types A-C. Shelter types used during the experiment to investigate shelter preferences of *Astacus astacus*. Tubes (A), block-filter (B) and wool-filter (C).

of the crayfish during the night. Juvenile crayfish (age 1 year) were obtained from a pond culture (Göckemeyer; Poggenhagen, Germany) and were grown for another year in a RAS. Each compartment was stocked with 12 size-graded juvenile Noble crayfish with a mean (\pm SD) weight 3.02 ± 1.35 g and mean carapace length of 23.3 ± 2.9 mm. The size classes were normally distributed within each compartment and are defined by carapace length as following: size class 1: $18 \leq x < 22$ mm; size class 2: $22 \leq x < 26$ mm; size class 3: $26 \leq x < 30$ mm. In order to maintain the same density in the compartments dead crayfish were replaced with crayfish of same sex and similar size. A commercial crayfish diet (Sera crabs natural, Sera GmbH, Heinsberg, Germany) was distributed in each compartment equally three times per week in the afternoon. The feeding rate based on a percentage of biomass, observed in earlier experiments of 2.5% per day. Therefore, all crayfish were measured before starting the experiment.

Data sampling

The numbers of the crayfish per shelter and outside were recorded daily in the morning over four weeks with 20 sampling days. In order to determine personal preference for shelter types each crayfish was marked individually and removed from the shelters every second day during the first ten sampling days. The position of each individual crayfish was recorded and the crayfish was repositioned in the shelter. Additionally, moulting was checked daily and moulted crayfish were weighed, measured (carapace length) and remarked at the next sampling day.

Data treatment and statistical analysis

The shelter preference given as percentage of total number of individuals was calculated with formula:

1. Shelter preference (%) = $100 \times \text{sum of counts in shelter} / \text{sum of total counts in compartment}$

A three-way ANOVA was performed to test the shelter types and the effects of the compartments and the day of incubation followed by a Tukey test.

The size class dependent shelter preference given as the frequency (%) of the size classes (i) occurring in a shelter was calculated with formula:

2. Frequency (%) = $100 \times \text{sum of individuals in size class } i \text{ in shelter} / \text{sum in total number of individuals in size class } i$

A one-way ANOVA was performed for the size classes for each shelter type followed by a Tukey test. When normality test (Shapiro-Wilk) failed data were *arcsin* transformed.

An individual shelter preference was assumed if an individual spent 80% of experimental time or more during the 20-day trial in a specific shelter since crayfish are night active and stay if non-disturbed most of the day-time in one shelter. The individual shelter preference (%) given as percentage of total number of individuals was calculated with formula:

- Individual shelter preference (%) = $100 \times \frac{\text{number of individuals with 80\% or more time spent in a specific shelter}}{\text{sum of total counts in compartment}}$

As Normality test (Shapiro-Wilk) failed for the block-filter, wool-filter and outside data these data were arcsin transformed. Normality test for the wool-filter and outside data failed again, so Kruskal-Wallis One Way Analysis on ranks was performed.

Results

Shelter preference

Astacus astacus showed a clear shelter preference (three-way ANOVA: $F = 181.831$, $p < 0.001$), which was not affected by either experimental compartment or experimental day (tank $F = 0.00000357$, $p = 1.0$; day $F = 0.00000802$, $p = 1.0$). Frequency is shown as a percentage of the total number (four tanks pooled) harvested (Fig. 2). The highest percentage ($47.6 \pm 1.3\%$) of individuals preferred tubes as shelter during an experimental period of 20 days. A significant lower number of individuals preferred wool-filter and block-filter as shelter. On average, $21.5 \pm 3.6\%$ and $23.8 \pm 4.9\%$ of individuals occupied wool-filter and block-filter, respectively. Only $6.9 \pm 1.8\%$ of individuals did not use any type of shelter and could be recorded outside.

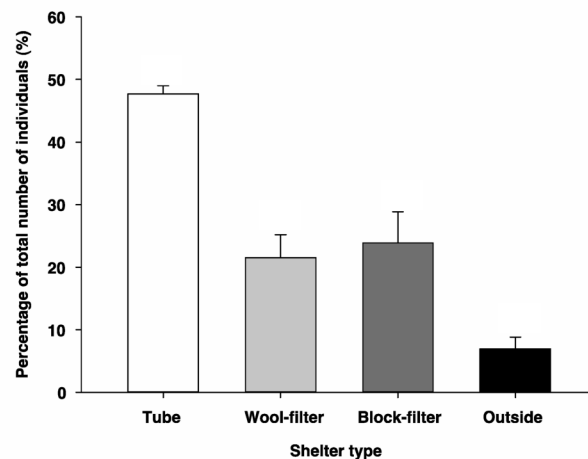


Figure 2 Shelter preference of *Astacus astacus* for tube, wool-filter or block-filter given as percentage of total number of individuals (%) \pm SE.

Size class dependent shelter preference

Individual size class had a strong effect on shelter preference (Fig. 3). While only $7.6 \pm 10.1\%$ of animals of size class 1 preferred tubes as shelter, a significant higher number of size class 2 ($56.5 \pm 28.5\%$) and size class 3 ($78.1 \pm 18.8\%$) used tubes as shelter (one-way ANOVA, $F = 32.979$, $p < 0.001$) (Fig. 3 tube).

Size class also had a significant effect on the use of wool-filter (Kruskal-Wallis One Way Analysis, $p = 0.002$) as well as block-filter as shelter (one-way ANOVA $F = 4.204$, $p = 0.022$).

Wool-filter was preferred by $33.4 \pm 19.5\%$ of size class 1 animals, while only $9.2 \pm 13.9\%$ of size class 2 and $12.7 \pm 16.1\%$ of size class 3 individuals were regularly found in this shelter type (Fig. 3 wool-filter). Almost equal numbers of size class 1 ($36.9 \pm 22.5\%$) and size class 2 ($32.2 \pm 33.2\%$) individuals preferred block-filter as shelter (Fig. 3 filter). Block-filter as shelter was used less frequently by size class 3 individuals ($9.0 \pm 13.7\%$). Size class of individuals significantly affected the time spent outside the shelters (Kruskal-Wallis One Way

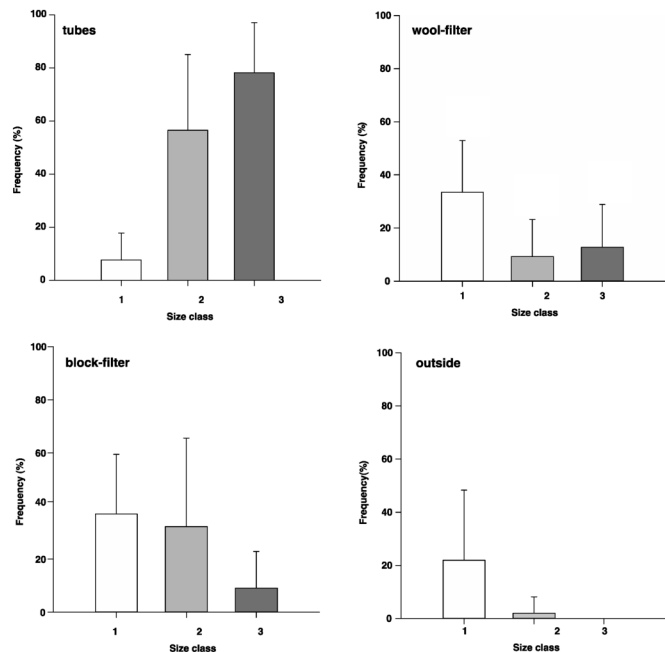


Figure 3 Shelter preferences of different size classes (1, 2 and 3) of *Astacus astacus* for tubes, wool-filter or block-filter given as frequency (%) \pm SE.

Analysis, $p = 0.001$). However, a *posteriori* analysis revealed no significant difference between size classes (Fig. 3 outside). $21.9 \pm 26.5\%$ of size class 1 individuals regularly spent time outside the shelters, while no size class 3 animals and only $2.0 \pm 6.1\%$ of size class 2 animals were recorded outside. A relation of the four dead animals to a specific shelter or group was not identified.

Individual preference

Around 41% of all individuals showed an individual shelter preference with 80% or more of time during the 20-day trial. $32.1 \pm 7.1\%$ of these animals preferred tubes as shelter, while $9.3 \pm 12.6\%$ regularly used block-filter as shelter (Fig. 4).

However, there was no significant difference (One Way Repeated Measures Analysis of Variance, $F = 7.741$, $p = 0.069$) in their individual preferences. Also, no animal showed an individual preference for wool-filter as shelter.

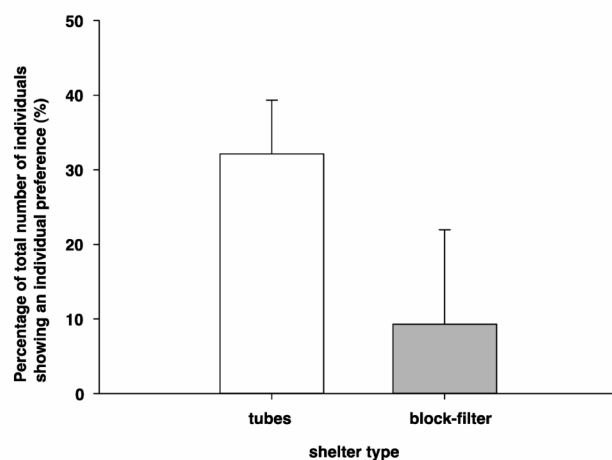


Figure 4 Individual shelter preferences of *Astacus astacus* for tubes or block-filter given as percentage of total number of individuals showing an individual preference (%) \pm SE.

Discussion

The overall result of this experiment presents tubes as the preferred shelter type although more complex structures were expected to be preferred due to foothold and naturalness. On the contrary, these tubes might have offered a better shelter accessibility and the tube form could have met the demands of the crayfish better than the block and wool filter regarding handling and defense. These more complex structures featured better shelter conditions against less accessibility. In addition, wool-filter as well as outside counted crayfish might have offered foraging opportunities for smaller crayfish against the bigger and therefore superior crayfish. An additional experiment using different crayfish ages is necessary to examine this assumption and give insight in crayfish requirements for their shelter choice.

A classification of the used two-year-old crayfish into size class groups offered further insight into shelter preference and showed that shelter type preference differed in dependence of length. Smaller crayfish preferred more complex structures like block- and wool-filter against simple tubes while with increasing length, crayfish longer than 22 mm chose tubes as shelter. These longer crayfish also stayed constantly in their shelter over the day and seemed to develop an individual shelter preference. On the contrary, smaller animals showed a larger span and variance regarding day activity and shelter preference which was also observed in other experiments (Seemann, unpublished data).

The day activity of these animals could be linked to the light exposed wool filter structure which could have resulted in an unequivocally higher activity of size class 1 animals. Using wool filter therefore indicates a possibility to influence the natural day/night activity of these crayfish and could present a possibility to enhance (or reduce) crayfish growth.

According to the experiment results, shelter types did not influence survival rate of crayfish. Whether different shelters affect growth for this crayfish age can be answered when more data is available for a longer growth period. If shelter types are applied in crayfish farms, different diameters (tube, block-filter) or densities (wool-filter) have to be supplied for different crayfish sizes.

Conclusion

The three tested materials proved to be suitable for RAS since they are light, easy to handle and can be proceeded with moderate costs in considerable amounts. Regarding a commercial grow-out system, shelter types should be altered in dependence of the crayfish age and length. As increasing crayfish length can be seen as a handicap for shelter handling, structures like block- and wool-filter should be used for animals of sizes less than 22 mm carapax length, which are able to handle these fine-macromesh structures. In addition, wool-filter can reduce competition for feed and offers immediate shelter access from all directions after moulting, even for longer crayfish. Better feed access also supports smaller crayfish to reach standard

growth rates due to prior disadvantages in feed struggle. Simpler structures like tubes should be used for longer crayfish according to their growing moving-handicap in fine structures. Research questions regarding numbers of shelters per individual and age and best fitting densities and diameters of shelters depending on crayfish size still have to be answered. Also, the influence of shelter types on growth rates has to be investigated.

References

- Celada, J. D., Carral, J. M., Gaudioso, V. R., González, J., Lopez-Baissón, C., & Fernández, R. (1993). Survival and Growth of Juvenile Freshwater Crayfish *Pacifastacus leniusculus* Dana Fed Two Raw Diets and Two Commercial Formulated Feeds. *Journal of the World Aquaculture Society*, 24(1), 108-111. doi:10.1111/j.1749-7345.1993.tb00157.x
- Franke, R., Wessels, S., & Horstgen-Schwark, G. (2011). Enhancement of survival and growth in crowded groups: the road towards an intensive production of the noble crayfish *Astacus astacus* L. in indoor recirculation systems. *Aquaculture Research*, 44(3), 451-461. doi:DOI 10.1111/j.1365-2109.2011.03052.x
- Gydemo, R., & Westin, L. (1989). Growth and survival of juvenile *Astacus astacus* L. at optimized water temperature. In N. De Pauw & e. al. (Eds.), *Aquaculture: a biotechnology in progress* (Vol. 1, pp. 383-391). Bredene, Belgium: European Aquaculture Society.
- Hager, J. (2003). *Edelkrebse - Biologie, Zucht, Bewirtschaftung* (2., überarbeitet Auflage ed.). Graz - Stuttgart: Leopold Stocker Verlag.
- Harlioğlu, M. M. (2007). The harvest of the freshwater crayfish *Astacus leptodactylus* Eschscholtz in Turkey: harvest history, impact of crayfish plague, and present distribution of harvested populations. *Aquaculture International*, 16(4), 351-360. doi:10.1007/s10499-007-9145-7
- Holdich, D. M. (1993). A review of astaciculture: freshwater crayfish farming. *Aquatic Living Resources*, 6(4), 307-317.
- Holdich, D. M., & Lowery, R. S. (1988). *Freshwater crayfish : biology, management and exploitation*. London [etc.]: Croom Helm [etc.].
- Jones, C. M. (1995). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae) I. Development of hatchery and nursery procedures. *Aquaculture*, 138(1-4), 221-238. doi:http://dx.doi.org/10.1016/0044-8486(95)00068-2
- Jones, C. M., & Ruscoe, I. M. (2001). Assessment of Five Shelter Types in the Production of Redclaw Crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae) Under Earthen

- Pond Conditions. *Journal of the World Aquaculture Society*, 32(1), 41-52. doi:10.1111/j.1749-7345.2001.tb00920.x
- Keller, M. (2017). Die Rolle der Krebse in früheren Jahrhunderten. *forum flusskrebse - Historisches zum Flusskrebs*.
- Kozák, P., Füreder, L., Kouba, A., Reynolds, J., & Souty-Grosset, C. (2011). Current conservation strategies for European crayfish. *Knowledge and Management of Aquatic Ecosystems*(401), 01. doi:10.1051/kmae/2011018
- Martin, A. L., & Moore, P. A. (2008). The influence of dominance on shelter preference and eviction rates in the crayfish, *Orconectes rusticus*. *Ethology*, 114(4), 351-360.
- Martins, C. I. M., Eding, E. H., Verdegem, M. C. J., . . . J. A. J. (2010). *New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability* (Vol. 43). Amsterdam, PAYS-BAS: Elsevier.
- Mitchell, B. D., Collins, R. O., & Austin, C. M. (1994). Multi-level refuge utilization by the freshwater crayfish *Cherax destructor* Clark (Decapoda:Parastacidae): a potential harvest and sampling technique. *Aquaculture Research*, 25(5), 557-562. doi:10.1111/j.1365-2109.1994.tb00719.x
- Pöckl, M. (1998). Verbreitung und Ökologie in Österreich vorkommender Flußkrebse. *Stapfia*, 58, 119-130.
- Rabeni, C. F. (1985). Resource Partitioning by Stream-dwelling Crayfish: The Influence of Body Size. *American Midland Naturalist*, 113(1), 20-29.
- Sáez-Royuela, M., Carral, J. M., Celada, J. D., & Pérez, J. R. (2001). Effects of shelter type and food supply frequency on survival and growth of stage-2 juvenile white-clawed crayfish (*Austropotamobius pallipes* Lereboullet) under laboratory conditions. *Aquaculture International*, 9(6), 489-497. doi:10.1023/a:1020509627870
- Seemann, U. B., Lorkowski, K., Schiffer, M., Hörterer, C., Slater, M. J., & Buck, B. H. (2014). Survival of early stripped eggs of the noble crayfish *Astacus astacus* and effects of saline solution during artificial incubation. *Freshwater Crayfish*, 20(1), 1-6. doi:10.5869/fc.2014.v20-1.1
- Seemann, U. B., Lorkowski, K., Slater, M. J., Buchholz, F., & Buck, B. H. (2014). Growth performance of Noble crayfish *Astacus astacus* in recirculating aquaculture systems. *Aquaculture International*, 23(4), 997-1012. doi:10.1007/s10499-014-9859-2
- Skurdal, J., & Taugbøll, T. (1994). *Biology, Culture and Management of the Noble Crayfish Astacus Astacus L.*
- Söderbäck, B. (1994). Interactions among juveniles of two freshwater crayfish species and a predatory fish. *Oecologia*, 100(3), 229-235. doi:10.1007/bf00316949

- Taugbøl, T., & Skurdal, J. (1992). Growth, mortality and moulting rate of noble crayfish *Astacus astacus* L., juveniles in aquaculture experiments. *Aquaculture and Fisheries Management*, 23, 411-420.
- Zimmermann, J. K. M. (2012). *Noble Crayfish (Astacus astacus) in a Changing World – Implications for Management*. (Doctor Doctor), Mid Sweden University, SE - 851 70 Sundsvall, Sundsvall. Sweden. (126)

4. Synthesis and Perspectives

Over 3 ½ years these aspects were studied in different work packages in RAS and OPS. In the Centre for Aquaculture Research (ZAF) of the Alfred Wegener Institute for Polar and Marine Research (AWI) several systems were designed and tested for these purposes and used for feeding and breeding experiments. Data from a trout and Noble crayfish farm were collected over two years to compare it with its performance in a recirculating aquaculture system (RAS) and give hints for improvements. Growth rates of *A. astacus* in RAS were measured over several years using different industrial feeds and compared with data from the open pond system. Energy, Protein, total fat and fatty acid analysis of feed and crayfish were performed to give further conclusions. A variety of materials was tested for shelter suitability and Red Nile tilapia *Oreochromis niloticus* exemplarily used as a co-culture species in polyculture experiments.

4.1. Growth performance and feed

Astacus astacus is an omnivorous but specific feeder focusing on invertebrates, plants and detritus (Skurdal, Taugbøll, 1994). While the results of the first feeding experiment showed higher growth rates in an OPS, particularly during the high season, overall annual growth in recirculating aquaculture system (RAS) is higher. As feed is the crucial growth factor to provide all nutrients needed for best growth an industrial and affordable crayfish feed is mandatory for an economic crayfish culture in RAS. While Ackefors, Castell, Boston, Rätty, Svensson (1992) already stated lipid (<10%), carbohydrate (20-25%) and protein (25%) proportions for a commercial diet formulation for young crayfish, the fatty acid (FA) composition and amount also play an essential role in achieving high growth rates as investigated in this study. If not provided in sufficient variety and amount Noble crayfish synthesize FA themselves and lose energy needed for growth. After several trials with other industrial feeds for trout and shrimp self-manufactured feeds provided among others all 18 FA in considerable amounts according to the findings of (Seemann, Lorkowski, Slater, Buck, Buchholz, 2017). These feeds finally allowed even slightly better growth rates than OPS crayfish.

If need rises for an industrial crayfish feed the resource origin of the composites should come from sustainable sources to reduce e.g. fishmeal produced of wild-caught fish. Referring to this our results suggest that both *Elodea spec.* and mussel meal of *Mytilus edulis* can be used

in crayfish production as a basis for an extruded feed or at least as a supplementary diet if economically viable (D'Agaro, 2006; D'Agaro, Renai, Gherardi, 2004; Seemann, Lorkowski, Slater, Buck, Buchholz, 2017). An economic study is necessary to evaluate diet resource origins and production costs of such a commercial diet and draw comparisons to mixtures of industrial feed at that time.

Furthermore, high survival rates and constant conditions are advantageous for crayfish culture in RAS. As showed, one-year old Noble crayfish can be cultured at high stocking densities up to 300 individuals per m². Density trials with three-year old crayfish with 100 % survival with ten individuals per m² over two weeks are promising for crayfish culture as well. To optimize average growth it is suggested to grade crayfish at least every six month to reduce inhibitory effect of larger dominant individuals on smaller ones as indicated from other studies as well (Ackefors, Castell, Örde-Öström, 1997; Ahvenharju, Savolainen, Tulonen, Ruohonen, 2005; Franke, Hörstgen-Schwark, 2015; Jähne, 2014). Equally, losses due to moult-induced cannibalism would be prevented. A grading in OPS is much more difficult and linked with a high work load. While impossible for summerlings and one-year old crayfish as these animals are too small to find in an OPS it is suggested for two and three-year-old crayfish.

4.2. Reproduction

A complete reproduction in RAS is possible and a good opportunity to raise survival rates in regard to high losses in OPS even if done in separate tanks. Disturbances like noise and vibrations from pumps and maintenance work as well as light fluctuations of non-dimmed illumination can easily interrupt mating process and even lead to loss of eggs while attached to the females. Since eggs are more sensibly after ovulation and visual control it is not recommended at this time and all precautions should be taken to minimize stress, such as reduced daily routines, light dimming and shading. Also the detaching off eggs and the following daily egg control must be done very carefully over the period of 1.200 degree days (Hessen, Taugbøl, Fjeld, Skurdal, 1987). A relatively

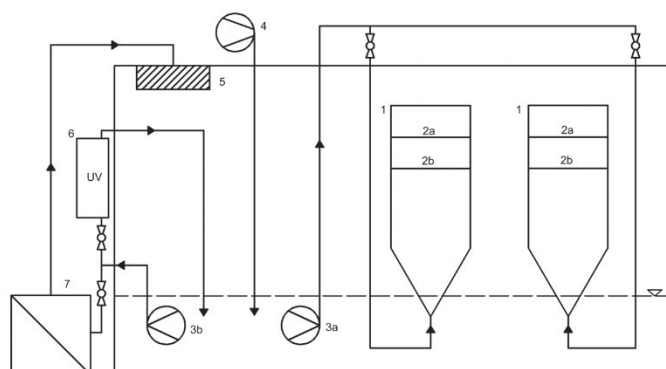


Figure 5 System design for artificial incubation of noble crayfish eggs. 1, Zougg jars; 2, Sieves with egg batches; 3, Pumps; 4, Air compressor; 5, mat and fine filter; 6, UV-filtration unit; 7, Cooling aggregate.

simple water recirculating unit suffices to avoid fungal infections including a saline bath of ten minutes every day for egg treatment (Fig. 5 and 6). A five-minute bath even increases survival rate (Seemann, Lorkowski, Schiffer, Hörterer, Slater, Buck, 2014). A water treatment unit is not mandatory but recommended for safety reasons. An advanced egg treatment unit was developed after the first experiment to allow better egg handling and daily work routines (Fig. 7). The small compartments allow individual

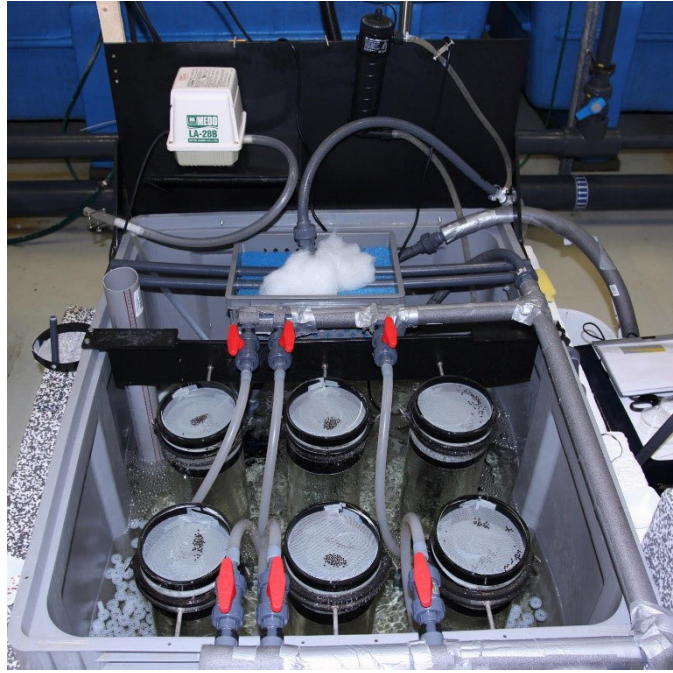


Figure 6 Artificial incubation unit for Nobel crayfish eggs as described in Fig 5.

monitoring of each egg while the mounting allows constant egg movement in the water column. The movement pattern is adopted from that of female crayfish. Some years of experience are necessary to maintain high and constant hatching rates. It is also necessary to identify the earliest time for egg-stripping by excluding or identifying variables like temperature gradient, broodstock feed, reproduction time, egg quality and system functionality in regard to production demands. The most demanding question is whether a reproduction twice or more per year is possible and which are the crucial key parameter since the shortening of egg development is already possible (Seemann, Lorkowski, Schiffer, Hörterer, Slater, Buck, 2014; Westin, Gydemo, 1986). Thus, far more research questions could be answered since reproduction is the time limiting factor in Noble crayfish research. Since the eradication of the crayfish plague is not possible due to its global saturation and its symbiosis with non-native crayfish species only genetic breeding programs for plague resistant crayfish strains might pose a solution. This afford will take a bulk of research but

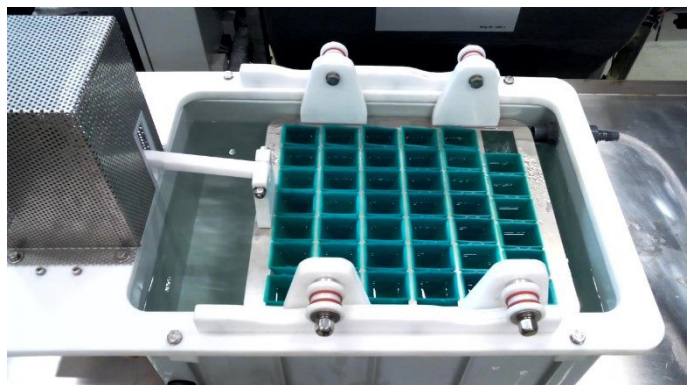


Figure 7 Advanced incubation unit containing individual compartments which are moved in vertical direction. This induces a horizontal shift of the eggs for better turbation and oxygen supply. The water treatment unit is similar to the previous version (Fig. 6).

propose the only perspective for a survival of Noble crayfish in the long-term beside restocking programs in secluded areas in the mid-term.

4.3. System and technique

According to the study two OPS and two RAS would be necessary for a complete reproduction cycle. First, a RAS or an OPS for mating and breeding is needed as it was used in the ZAF (Seemann, Lorkowski, Slater, Buchholz, Buck, 2014). A RAS would allow selected breeding and monitoring of individuals, which is recommended especially in regard to genetic diversity aspects like safeguard of autochthone populations and genetic strains (Schrimpf, Theissing, Dahlem, Maguire, Pârvulescu, Schulz, Schulz, 2014; Schrimpf, Piscione, Cammaerts, Herman, Collas, Jung, Ottburg, Roessink, Rollin, Schulz, Theissing, 2017). Second, a breeding system based on RAS like the presented egg breeding machine would guarantee constant hatchlings with adequate experience (Seemann, Lorkowski, Schiffer, Hörterer, Slater, Buck, 2014). Third, a grow-up RAS for young crayfish until transfer into a fourth OPS, where they grow up to harvest size, and finally an OPS, where the parental brood stock can be stocked for selective breeding.

Shelter solutions for crayfish in RAS must fulfill several characteristics. Easy cleaning and different sizes for crayfish sizes go along with the availability in high quantities and low production costs. No adequate shelters are currently not available on the market. The project results show that for small crayfish several industrial products are applicable e.g. trickling filter material and filter wool. As increasing crayfish size can be seen as a handicap for shelter handling, structures like wool-filter should be used for animals of less than 22 mm carapace length. These are able to handle these fine-macromesh structures while bigger animals are hampered in moving through the wool which reduces predatory losses (Seemann, Kröncke, Slater, Buck, 2017). In addition, wool-filter can reduce competition for feed and offers immediate shelter access from all directions after moulting, even for bigger crayfish. Better feed access also enables an evenly growth and supports smaller crayfish to catch up due to prior disadvantages in feed struggle. Simpler structures like tubes should be used for longer crayfish according to their growing handicap (Seemann, Lorkowski, Slater, Buck, Buchholz, 2017). The filter PVC pipes used in this study proved to be the best solution for all crayfish sizes as various diameters and lengths are available. They are easy to handle, durable, light, can be glued to each other even at different levels and are easy to clean. Since a variety of different materials and sizes were used in past research projects to fulfill shelter requirements

but without focus on their impact on e.g. growth and survival an exchange in knowledge and experiences on shelter efficiency would be helpful. The interpretation of these additional research outcomes would lead to best-fit shelter designs according to crayfish size and age and resolve an aggravating data situation. A professional mass production of such shelter batteries would be needed to provide a sufficient amount for research institutes and crayfish farms producing several tons of crayfish, especially since three shelters are needed per animal. A review about past research projects on exact numbers of shelters per individual and age and best stocking densities and diameters of shelters depending on crayfish size seems likely the best way to answer these questions.

4.4. Polyculture

The general potential of finfish concerning the utilization of incurred solids, in form of uneaten feed and feces, is high. Tilapia minimized the incurred solids of *A. astacus* and proved any additional tank cleaning redundant in the polyculture experiments. Thereby, the effective utilization of solids from Noble crayfish was raised. Growth and survival rates were not affected by the presence of tilapia even if in direct contact without further enclosures when finfish and crayfish are combined in a reasonable size. In general, the density of the crayfish should be according to their age and available m². Finfish number is dependent of their use as a) a supporting species in the same system and tanks refeeding on crayfish feed and effecting a positive biological rearrangement or b) cultured in separate tanks at high densities. Even a combination would be possible and reasonable in case b). If they are primary used as tank cleaner and bio deposit converter densities could be a few animals per m³ depending on crayfish size. Depending on the water level, which could be around 0.4m for monocultured crayfish only finfish of adequate size should be used. If finfish are the primary species in the system, like e.g. in a farm with salmon trout of 100 kg/m³, crayfish play a minor part due to economic value of salmon trout and moderate yield increase by polyculture with Noble crayfish. Since in Germany Noble crayfish aquaculture is limited to small OPS but several big trout farms exist with seven-digit turnovers the integration of crayfish must fit to the given restrictions of these already economic systems. The project IDeA (Integration of Detrivores in existing Aquaculture systems, AZ 31971/01) of the Aquaculture Research Group from the AWI works on identifying technical and economic issues of such an integration attempt by using tanks in a bypass and feed the crayfish with biomass from the farm.

4.5. Conservation, Economics and Sustainability

Breeding and restocking programs are obligatory for a survival of Noble crayfish. RAS offer the safest and secure option in regard to survival and plague risks but at high costs. The conservation of its different genetic strains as proposed by Schrimpf, Piscione, Cammaerts, Herman, Collas, Jung, Ottburg, Roessink, Rollin, Schulz, Theissing (2017) and Groß (2015) can only be guaranteed in RAS while OPS lacks in this safety aspect but is economically more attractive. As translocation and non-controlled breeding already led to low haplotype diversity in *A. astacus* for central Europe concentrated conservation management plans are necessary for this species (Schrimpf, Schulz, Theissing, Pârvulescu, Schulz, 2011). As in Switzerland a federal crayfish office already was established, other countries should follow to bundle conservation efforts for indigenous crayfish in Europe. A central breeding facility for known genetic and local strains would be an option to concentrate efforts, funded by European countries. This mutual project could start with a demonstration plant for several genetic strains and even different ICS. At the same time further research would be necessary for enhanced breeding cycles within one year to reduce seasonal reproduction limits like already done for *Cherax quadricarinatus* (Karplus, Gideon, Barki, 2003).

Due to economic reasons especially in regard to cost structures in Germany a mono-culture of Noble crayfish in RAS is not recommendable. This is due to relatively low densities of adult crayfish and particularly to long grow-out periods of approximately three to four years from hatch to harvest in comparison with other high value crustacean species like Whiteleg shrimp *Litopenaeus vannamei*. These shrimps are cultured up to seven kg/m² and only need five to six month to harvest (Rajkumar Singh, Raja, Gopalakrishnan, Kannan, Sakthivel, 2013), which is economically more attractive to investors with around five million EUR investment costs for 30 tons/year. A comparable crayfish farm would need an investment of around 25 million EUR with high amounts for land and hall space (Seemann, 2015). Hence, high investment and maintenance costs do not make a complete RAS culture profitable at this time. A viable solution would be a RAS for a) egg breeding and treatment and b) grow-up of juveniles to maximize survival rates of eggs and juveniles. The reproduction and grow up of hatchlings for 6-18 month would need considerable low space in comparison to a grow-out system for adult crayfish. Less workload and maintenance costs will reduce running costs significantly. Furthermore, such a system layout could concentrate on the reproduction of genetic strains, which is of high interest of European governments and supported by different governmental

and private funding instruments. This kind of crayfish ark could also be used for other endangered crayfish species needs since general crayfish requirements are equivalent and the adaptations to their specific needs are limited. Even the breeding of plague resistant crayfish is possible and might play a major role since the crayfish plague spreads without crayfish (Vaeßen, Groß, Nowak, 2016; Waldmann, 2019).

4.6. The Future of Noble Crayfish – A Perspective

While recent and today's projects only allow survival of Noble crayfish in enclosed facilities in the long term an action plan for survival of this species in nature and open ponds systems have to be approached as demanded by various experts (Westman, Savolainen, 2001). Ultimately, the use of OPS is not an option but mandatory for a sustainable and economically feasible reproduction of Noble crayfish and its remaining genetic strains due to low stocking densities of adult crayfish. Only RAS and OPS together offer a viable solution. RAS should be used for egg breeding and hatching while OPS over enough and affordable space for crayfish broodstocks and grow out phase. Since the OPS have to be limited in size to reduce the risk of a crayfish plague introduction, such a system would only work for small farms or for the conservation of ICS species and genetic strains.

The eradication of the crayfish plague is not seen as a practicable option. With genome editing technologies, it would be possible to identify and slice genetic information relevant for plague resistance from NICS and insert these DNA sequences into ICS crayfish with the help of genome editing enzymes. But financing costs, environmental laws and restrictions are in the way of such an approach. To protect ICS crayfish and reintroduce them in their natural habitats without being prone to plague infection other efforts can be made to breed plague resistant crayfish, especially since sightings of plague resistant Noble crayfish populations occurred recently. For such a breeding program several years of research have to be taken into account especially due to reproduction and breeding cycle of Noble crayfish (Taugbøl, Skurdal, 1990). Therefore, the shortening of the one-year reproduction cycle have to be focused on simultaneously (Franke, Hoerstgen-Schwark, 2013). The applied egg treatment and the shortening of the time for incubation together with high survival rates support these efforts (Seemann, Lorkowski, Schiffer, Hörterer, Slater, Buck, 2014). It must be taken into account that at least four different strains of *Aphanomyces astaci* are known until now (OIE, 2019). This implies that even an ICS crayfish population with a resistance for one strain can be

affected by the other ones albeit with a less dramatic course of disease. Individual report envision better and worse prospects for conservation efforts in general like a new fungus type, other decapoda acting as plague carrier or a latent infection in a robust Noble crayfish population (Jussila, Makkonen, Vainikka, Kortet, Kokko, 2011b; Kozubíková, Viljamaa-Dirks, Heinikainen, Petrusek, 2011; Tilmans, Mrugała, Svoboda, Engelsma, Petie, Soes, Nutbeam-Tuffs, Oidtmann, Roessink, Petrusek, 2014)

Since a breeding program would not be viable for all genetic strains only one should be selected to establish a plague resistant parental broodstock. This would allow interbreeding with other genetic strains to maintain not only one but also other strains. An essential precondition is that the selection should be done in regard to lowest impact on genetic information of autochthone strains and thus maintains biodiversity and a healthy gene pool. Before restocking the use of in RAS hatched crayfish should be investigated. Proper re-introduction measures have to be implemented if results show lower survival due to e.g. predatory pressure or inability of shelter and feed adoption. If the plague threat is overcome and a stable production of Noble crayfish established the return of Noble crayfish is a potential perspective in natural water bodies since invasive species are not superior to Noble crayfish in species-species interactions. The advantage of faster reproduction cycles and growth rates of invasive crayfish species can be counterbalanced with appropriate restocking rates of Noble crayfish and fishing of invasive crayfish, which would also present an economic value. "Protection by use" of this high value product Noble crayfish could be an appropriate slogan for this long-term project.

Independent of conversation aspects, the integration of Noble crayfish as a byproduct in extensive trout farms might be of interest with respect to higher overall yields. Whether such integration is feasible and economically reasonable has to be considered in regard to production type and the additional benefit of the crayfish. This question is subject of a DBU funded project at the AWI (AZ31971/01).

5. Conclusions

The various topics addressed in this thesis led to new findings about the potential of Noble crayfish culture in recirculating aquaculture systems (RAS) and open pond system (OPS). RAS proved generally suitable in regard to crayfish requirements. Even if not all of these are fully understood in detail an equal and in parts even slightly better growth rate could have been achieved in comparison to OPS. A crucial point in this achievement was the underestimated feed composition. A high-grade feed composition was used to achieve optimal growth performance while nutrient requirements still have to be investigated further in regards to e.g. crayfish age, protein and vitamin amounts and compositions. Neither one of the tested seven industrial feed for carp, trout and shrimp proved to be applicable why a production of new feed compositions comes to the fore linked with the identification of costs, resources origins and need. Reproduction of Noble crayfish in RAS is possible too and comes together with several advantages like egg control, reduced reproduction time and high survival rates. In regard to the actual status of endangered crayfish a breeding facility for autochthone strains seems the only opportunity to maintain the existing genpool and diversity at the moment. Such a facility would also offer the opportunity to breed plague resistant strains in the long-term. As shelters, low-cost PVC tubes proved suitable for RAS and industrial production since they are lightweight, easy to clean, available in different diameters and sizes and can be stuck together as shelter-batteries for better handling. The combination of RAS and OPS to avoid low densities of adult crayfish in expensive RAS seems to be an economic option for a monoculture but unreliable for investors since other species offer higher yields and a shorter return of invest. Polyculture for crayfish is possible and with regard to economic and technical requirements recommended for Noble crayfish. While crayfish itself are not affected by finfish co-culture the removal of solids from the tank bottom is no longer required. Crayfish can feed on non-eaten finfish feed and solid feces and do not reduce space required for finfish as shown for the exemplary polyculture with Red Nile tilapia. An adequate grading of crayfish in monoculture and co-cultured species in polyculture is mandatory to minimize predatory losses.

Referenzen

- Ackefors, H., Castell, J., Örde-Öström, I.L., 1997. Preliminary results on the fatty acid composition of freshwater crayfish, *Astacus astacus* and *Pacifastacus leniusculus*, held in captivity. *Journal of the World Aquaculture Society*. 28, 97-105.
- Ackefors, H., Castell, J.D., Boston, L.D., Rätty, P., Svensson, M., 1992. Standard experimental diets for crustacean nutrition research. II. Growth and survival of juvenile crayfish *Astacus astacus* (Linné) fed diets containing various amounts of protein, carbohydrate and lipid. *Aquaculture*. 104, 341-356.
- Ackefors, H.E.G., 2000. Freshwater crayfish farming technology in the 1990s: a European and global perspective. *Fish and Fisheries*. 1, 337-359.
- Ahvenharju, T., Savolainen, R., Tulonen, J., Ruohonen, K., 2005. Effects of size grading on growth, survival and cheliped injuries of signal crayfish (*Pacifastacus leniusculus* Dana) summerlings (age 0+). *Aquaculture Research*. 36, 857-867.
- Arle, J., Blondzik, K., Claussen, U., Duffek, A., Grimm, S., Hilliges, F., Kirschbaum, B., Kirst, I., Koch, D., Koschorreck, K., Lepom, P., Leujak, W., Mohaupt, V., Naumann, S., Pirntke, U., Rechenberg, J., Schilling, P., Ullrich, A., Wellmitz, J., Werner, S., Wolte, R., 2017. *Gewässer in Deutschland: Zustand und Bewertung*. Umweltbundesamt, Dessau-Roßlau.
- Arzbach, H.-H., 2010. *Biologie, Ökologie und Verhalten der Flusskrebse*. Krebsseminar Echem, Echem.
- Auvergne, A., 1979. Culture of crayfish, L'elevage des ecrevisses. Eds. du Point Veterinaire, Maisons-Alfort (France), pp. 87.
- Avery, J.L., Romaine, R.P., McClain, W.R., 1998. *Crawfish Production Production Economics, Pond Construction and Water Supply*. Southern Regional Aquaculture Center, 4.
- Axelsson, E., Nyström, P., Sidenmark, J., Brönmark, C., 1997. Crayfish predation on amphibian eggs and larvae. *Amphibia-Reptilia*. 18, 217-228.
- Bank, T.W., 2013. *Fish to 2030 Prospects for Fisheries and Aquaculture*. Agriculture and Environmental Services Discussion Washington.
- Barki, A., Karplus, I., Manor, R., Parnes, S., Aflalo, E.D., Sagi, A., 2006. Growth of redclaw crayfish (*Cherax quadricarinatus*) in a three-dimensional compartments system: Does a neighbor matter? *Aquaculture*. 252, 348-355.
- Basen, T., Chucholl, C., 2015. *Harte Schale, leckerer Kern - Krebse und Muscheln als Lebensmittel*.

- Behringer, D.C., 2012. Diseases of wild and cultered juvenile crustaceans. *Journal of Invertebrate Pathology*. 110, 225-233.
- Blohm, H.-P., Gaumert, D., Kämmereit, M., 1994. Leitfaden für die Wieder- und Neuansiedlung von Fischarten. Binnenfischerei in Niedersachsen. Niedersächsisches Landesamt für Ökologie, Hildesheim, pp. 90.
- Bong Chang, J., Lee, Y., 2018. The effects of technological development on fisheries production. *Fisheries Science*. 85.
- Boyd, C., A. McNevin, A., 2014. An Overview of Aquaculture. 1-20.
- Bregnballe, J., 2015. A Guide to Recirculating Aquaculture - An introduction to the new environmentally friendly and highly productive closed fish farming systems. FAO; EUROFISH, Copenhagen.
- Chopin, T., 2013. Aquaculture Aquaculture , Integrated Multi-trophic (IMTA) aquaculture integrated multi-trophic (IMTA). 184-205.
- Chucholl, C., 2012. Notorisch gefährdet: Probleme, Strategien und Perspektiven im Artenschutz für Edelkrebse, SVK-Fischereitagung, Fulda, pp. 18.
- Chucholl, C., Dümpelmann, C., 2017. Sondergutachten 2017: Erstellung einer Expertise zu Krebsperren und alternativen Schutzmaßnahmen für den Steinkrebs.
- Cukerzis, J.M., 1973. Biologische Grundlagen der Methode der künstlichen Aufzucht der Brut des *Astacus astacus* L. *Freshwater Crayfish*. 1, 187-201.
- Cukerzis, J.M., 1988. *Astacus astacus* in Europe. *Freshwater Crayfish. Biology*.
- D'Agaro, E., 2006. Utilization of Water Cress (*Nasturtium Officinale* L.) in Noble crayfish (*Astacus Astacus*) feeding. *Bulletin Français de la Pêche et de la Pisciculture*, 1255-1260.
- D'Agaro, E., Renai, B., Gherardi, F., 2004. Evaluation of the American waterweed (*Elodea Canadensis* Michx.) as supplemental food for the Noble crayfish, *Astacus astacus*. *Bulletin Français de la Pêche et de la Pisciculture*, 439-445.
- Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K., Pedersen, P.B., 2013. Farming different species in RAS in Nordic countries: Current status and future perspectives. *Aquacultural Engineering*. 53, 2-13.
- Daws, A.G., Grills, J., Konzen, K., Moore, P.A., 2002. Previous Experiences Alter the Outcome of Aggressive Interactions Between Males in the Crayfish, *Procambarus Clarkii*. *Marine and Freshwater Behaviour and Physiology*. 35, 139-148.

- Dethlefs, A., 2007. Predator - Prey - Interaction between Dragonfly Larva (Odonata: *Anisoptera*) and Crayfish Juveniles (*Astacus astacus*), Zoological Institute - Department Limnology. Christian - Albrechts - University Kiel, Kiel, Germany, pp. 124.
- Ebeling, M., 2014. Market prices for *Astacus astacus*. in: Seemann, U.B. (Ed.).
- Egerton, B.F., Evans, L.H., Stephens, F.J., Overstreet, R.M., 2002. Synopsis of freshwater crayfish diseases and commensal organisms. *Aquaculture*. 206, 57-135.
- Edsman, L., Füreder, L., Gherardi, F., Souty-Grosset, C., 2010. *Astacus astacus* - Status report, The IUCN Red List of Threatened Species. International Union for Conservation of Nature and Natural Resources.
- Espinal, C.A., Matulić, D., 2019. Recirculating Aquaculture Technologies. 35-76.
- FAO, 2012. Feeding the Growing Aquaculture Sector: An Analysis. in: *Aquaculture*, S.-C.o. (Ed.). FAO - Committee on Fisheries, Cape Town, South Africa.
- FAO, 2018. The State of World Fisheries and Aquaculture - Meeting the Sustainable Development Goals. Licence: CC BY-NC-SA 3.0 IGO.
- Fischereibehörde, S.L.f.L.-. 2012. Flusskrebse - Bestimmungsschlüssel.
- Franke, R., Hörstgen-Schwark, G., 2013. Lunar-rhythmic molting in laboratory populations of the Noble Crayfish *Astacus astacus* (Crustacea, Astacidea): an experimental analysis. *PLoS One*. 8, e68653.
- Franke, R., Hörstgen-Schwark, G., 2015. Influence of social factors on the nocturnal activity pattern of the noble crayfish, *Astacus astacus* (Crustacea, Decapoda) in recirculating aquaculture systems. *Aquaculture Research*. 46, 2929-2937.
- Franke, R., Wessels, S., Hörstgen-Schwark, G., 2011. Enhancement of survival and growth in crowded groups: the road towards an intensive production of the noble crayfish *Astacus astacus* L. in indoor recirculation systems. *Aquaculture Research*. 44, 451-461.
- Füreder, L., 2009. Flusskrebse Biologie - Ökologie - Gefährdung. Folio Verlag, Wien.
- Gherardi, F., 2011. Towards a sustainable human use of freshwater crayfish (Crustacea, Decapoda, Astacidea). *Knowl. Managt. Aquatic Ecosyst.*, 02.
- Göckemeyer, S., 2014. How to reduce extensive loss of juvenily crayfish in OPS. in: Seemann, U.B. (Ed.), Poggenhagen.
- Groß, H., 2015. Was bringen genetische Untersuchungen von Flusskrebsen für den Artenschutz?, forum flusskrebse. Forum Flusskrebse, Bad Münstereifel.

- Haase, T., Heidecke, D., Klapperstück, J., 1989. Zur Ökologie und Verbreitung des Edelkrebse *Astacus astacus* in der DDR Hercynia N. F. 26, 21.
- Hager, J., 2003. Edelkrebse - Biologie, Zucht, Bewirtschaftung, 2., überarbeitete Auflage ed. Leopold Stocker Verlag, Graz - Stuttgart.
- Hessen, D.O., Taugbøl, T., Fjeld, E., Skurdal, J., 1987. Egg development and lifecycle timing in the noble crayfish (*Astacus astacus*). *Aquaculture*. 64, 77-82.
- Holdich, D.M., 2002. Distribution of crayfish in Europe and some adjoining countries. *Bulletin Français de la Pêche et de la Pisciculture*, 611-650.
- Holdich, D.M., Reynolds, J.D., Souty-Grosset, C., Sibley, P.J., 2009. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowl. Managt. Aquatic Ecosyst.*, 11.
- Holdich, D.M., Reynolds, J.D., Souty-Grosset, C., Sibley, P.J., 2010. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems*, 11.
- Huner, J.V., 1994. Cultivation of freshwater crayfishes in North America. in: Huner, J.V. (Ed.), *Freshwater Crayfish Aquaculture in North America, Europe, and Australia*. The Haworth Press, New York, pp. 5-156.
- Hutchinson, W., Mathew, J., O'Sullivan, D.D., Casement, D., Clarke, S., 2004. *Recirculating Aquaculture Systems: Minimum Standards for Design, Construction and Management*. Inland Aquaculture Association of South Australia Inc., South Australia, Kent Town.
- Ingle, R., 1997. *Crayfishes, lobsters and crabs of Europe - An illustrated guide to common and traded species*. Springer.
- IUCN, 2019. *The IUCN Red List of Threatened Species*.
- Jähne, G., 2014. Kultur des europäischen Edelkrebse (*Astacus Astacus*) in landbasierten Kreislaufsystemen: Größenklassensortierung und technische Anpassungen zur Verbesserung der Kulturbedingungen, Aquakultur und Sea-Ranching. Universität Rostock, Rostock, pp. 153.
- Jeske, H., 2010. Edelkebs *Astacus astacus* - Zucht, Vermehrung und Bewirtschaftung von Edelkrebsen. Krebsseminar, Echem.
- Jover, M., Fernandez-Carmona, J., Del Rio, M., Soler, M., 1999. Effect of feeding cooked-extruded diets, containing different levels of protein, lipid and carbohydrate on growth of red swamp crayfish (*Procambarus clarkii*). *Aquaculture*. 178, 127-137.

- Jussila, J., Makkonen, J., Vainikka, A., Kortet, R., Kokko, H., 2011a. Latent crayfish plague (*Aphanomyces astaci*) infection in a robust wild noble crayfish (*Astacus astacus*) population. *Aquaculture*. 321, 17-20.
- Jussila, J., Makkonen, J., Vainikka, A., Kortet, R., Kokko, H., 2011b. Latent crayfish plague (*Aphanomyces astaci*) infection in a robust wild noble crayfish (*Astacus astacus*) population. *Aquaculture*. 321, 17-20.
- Karplus, I., Gideon, H., Barki, A., 2003. Shifting the natural spring–summer breeding season of the Australian freshwater crayfish *Cherax quadricarinatus* into the winter by environmental manipulations. *Aquaculture*. 220, 277-286.
- Keller, M., 2017. Die Rolle der Krebse in früheren Jahrhunderten, forum flusskrebse - Historisches zum Flusskrebs. Forum Flusskrebse, Bad Münstereifel.
- Kerth, G., Fischer, K., Fleischer, T., Limberg, J., Blüthgen, N., Dworchak, K., Dittrich, C., Rödel, M., Obermaier, E., 2015. Anpassungskapazität von 50 Arten mit potentiell hohem Austerberisiko gegenüber dem Klimawandel in Deutschland. *Natur und Landschaft*. 90, 17-24.
- Knudsen, H., Tveite, S., 1999. Survival and growth of juvenile lobster *Homarus gammarus* L. raised for stock enhancement within in situ cages. *Aquaculture Research*. 30, 421-425.
- Kozák, P., Buřič, M., Policar, T., Hamáčková, J., Lepičová, A., 2007. The effect of inter- and intra-specific competition on survival and growth rate of native juvenile noble crayfish *Astacus astacus* and alien spiny-cheek crayfish *Orconectes limosus*. *Hydrobiologia*. 590, 85-94.
- Kozák, P., Füreder, L., Kouba, A., Reynolds, J., Souty-Grosset, C., 2011. Current conservation strategies for European crayfish. *Knowledge and Management of Aquatic Ecosystems*, 01.
- Kozák, P., Duris, Z., Petrusek, A., Buric, M., Horka, I., Kouba, A., Kozubikova-Balcarova, E., Policar, T., 2015. *Crayfish Biology and Culture*. University of South Bohemia, Vodnany.
- Kozubíková, E., Viljamaa-Dirks, S., Heinikainen, S., Petrusek, A., 2011. Spiny-cheek crayfish *Orconectes limosus* carry a novel genotype of the crayfish plague pathogen *Aphanomyces astaci*. *Journal of Invertebrate Pathology*. 108, 214-216.
- Krieg, R., 2013. *Krebssperren in kleineren Gewässern*, Sissach.
- Krieg, R., Zenker, A., 2014. *Krebssperren: Feldversuche in einer Fischtreppe, einem natürlichen und einem verbauten Gewässer*. Fachhochschule Nordwestschweiz, Muttenz.
- Kühn, C., 2015. *Einbindung einer Edelkrebsaquakultur in ein integriertes Aquakultursystem am Standort Hohen Wangelin in den Jahren 2012 - 2015*. Institut für Fischerei, Rostock.

- LAVES, 2011. Vollzugshinweise zum Schutz von Krebsarten in Niedersachsen: Edelkrebs (*Astacus astacus*) - Niedersächsische Strategie zum Arten- und Biotopschutz. in: Binnenfischerei, D. (Ed.). Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit – LAVES, Hannover, pp. 12.
- Lennard, W., Goddek, S., 2019. Aquaponics: The Basics. 113-143.
- Maiwald, T., 2007. Verdrängung autochthoner Flusskrebarten durch invasive gebietsfremde Flusskrebarten in Mitteleuropa. in: Korn, H., Feit, U. (Eds.), Treffpunkt Biologische Vielfalt VII - Interdisziplinärer Forschungsaustausch im Rahmen des Übereinkommens über die biologische Vielfalt. BfN, Bonn, pp. 119-126.
- Manor, R., Segev, R., Leibovitz, M.P., Aflalo, E.D., Sagi, A., 2002. Intensification of redclaw crayfish *Cherax quadricarinatus* culture: II. Growout in a separate cell system. Aquacultural Engineering. 26, 263-276.
- McClain, W.R., 2012. Crawfish Production: Pond Construction and Water Requirements. in: Center, S.R.A. (Ed.), pp. 6.
- Möllers, F., 2013. Zwischenbericht Artenschutzprojekt „Edelkrebse in der Nette“ der NWA e.V. Niedersächsisch Westfälische Anglervereinigung e.V., Wallenhorst-Rulle.
- Murray, F., Bostock, J., Fletcher, M., 2014. Review of RAS technologies and their commercial application. Insitute of Aquaculture, Stirling.
- Murray, F., Bostock, J., Fletcher, M., 2018. An update on the 2014 report: “Review of Recirculation Aquaculture System Technologies and their Commercial Application”. Insitute of Aquaculture, Glasgow.
- O’Sullivan, D.D., Jones, C., Fielder, D., 2012. Freshwater Crustaceans. in: Lucas, J.S., Southgate, P.C. (Eds.), Aquaculture: Farming Aquatic Animals and Plants. Wiley-Blackwell, pp. 948 (420-442).
- Oidtman, B., Cerenius, L., Schmid, I., Hoffmann, R., Söderhäll, K., 1999. Crayfish plague epizootics in Germany - classification of two German isolates of the crayfish plague fungus *Aphanomyces astaci* by random amplification of polymorphic DNA. Diseases of Aquatic Organisms. 35, 235-238.
- OIE, 2019. Infection with *Aphanomyces astaci* (Crayfish plague), Manual of Diagnostic Tests for Aquatic Animals. The World Organisation for Animal Health (OIE), Paris, pp. 18.
- Palm, H.W., Knaus, U., Appelbaum, S., Strauch, S.M., Kotzen, B., 2019. Coupled Aquaponics Systems. 163-199.

- Peay, S., Füreder, L., 2011. Two indigenous European crayfish under threat – how can we retain them in aquatic ecosystems for the future? *Knowledge and Management of Aquatic Ecosystems*, 33.
- Peer, M., Pfeiffer, M., 2008. Erfassung der Bestandssituation der Flusskrebse im Freistaat Sachsen - Möglichkeiten für Schutz- und Entwicklungsmaßnahmen. Sächsisches Landesamt für Umwelt, Landwirtschaft und Geologie, Dresden.
- Perez Benavente, G., Uglem, I., Browne, R., Marino Balsa, C., 2010. Culture of juvenile European lobster (*Homarus gammarus* L.) in submerged cages. *Aquaculture International*. 18, 1177-1189.
- Phillips, M., 2018. Mariculture Overview.
- Pöckl, M., 1998a. Funktionelle Morphologie und Anatomie der Flußkrebse. Biologiezentrum Linz, Austria, Linz, pp. 131-142.
- Pöckl, M., 1998b. Nahrung und Ernährung der Flußkrebse. *Stapfia* 58. 137.
- Policar, T., Simon, V., Kozál, P., 2004. Egg Incubation in the Noble Crayfish (*Astacus astacus* L.): The Effect of Controlled Laboratory and Outdoor Ambient Condition on Hatching Success, Growth and Survival Rate of Juveniles. *Bulletin Francais de la Peche et de la Pisciculture*.
- Rajkumar Singh, R., Raja, K., Gopalakrishnan, A., Kannan, D., Sakthivel, A., 2013. Study on Growth Performance of *Litopenaeus vannamei* Cultured in Bore Well and Braksih Water Fed Ponds. *International Journal of Current Research*. 5, 3567-3570.
- Reynolds, J., Souty-Grosset, C., 2011. Management of Freshwater Biodiversity: Crayfish as Bioindicators. Cambridge University Press.
- Ridler, N., Wowchuk, M., Robinson, B., Barrington, K., Chopin, T., Robinson, S., Page, F., Reid, G., Szemerda, M., Sewuster, J., Boyne-Travis, S., 2007. Integrated Multi – trophic Aquaculture (Imta): A Potential Strategic Choice for Farmers. *Aquaculture Economics & Management*. 11, 99-110.
- Sander, M., 1998. Aquarientechnik im Süß- und Seewasser. Ulmer, Stuttgart.
- Schrimpf, A., Schulz, H.K., Theissing, K., Pârvulescu, L., Schulz, R., 2011. The first large-scale genetic analysis of the vulnerable noble crayfish *Astacus astacus* reveals low haplotype diversity in central European populations. *Knowledge and Management of Aquatic Ecosystems*, 35.
- Schrimpf, A., Pârvulescu, L., Copilas-Ciocianu, D., Petrussek, A., Schulz, R., 2012. Crayfish plague pathogen detected in the Danube Delta- a potential threat to freshwater biodiversity in southeastern Europe. *Aquatic Invasions*. 7, 503-510.

- Schrimpf, A., Theissing, K., Dahlem, J., Maguire, I., Pârvulescu, L., Schulz, H.K., Schulz, R., 2014. Phylogeography of noble crayfish (*Astacus astacus*) reveals multiple refugia. *Freshwater Biology*. 59, 761-776.
- Schrimpf, A., Piscione, M., Cammaerts, R., Herman, D., Collas, M., Jung, A., Ottburg, F., Roessink, I., Rollin, X., Schulz, R., Theissing, K., 2017. Genetic characterization of western European noble crayfish populations (*Astacus astacus*) for conservation management. *Conservation Genetics*. 18, 1299-1315.
- Seemann, U.B., 2015. Förderinitiative Aquakultur: Nachhaltige Besatzkrebiszucht in einem geschlossenen Aquakultur-Kreislaufsystem am Beispiel des bedrohten Edelkrebse „*Astacus astacus*“ <AZ28879_Abschlussbericht.pdf>. Alfred Wegener Institut Helmholtz Zentrum für Polar- und Meeresforschung, Bremerhaven.
- Seemann, U.B., Kröncke, N., Slater, M.J., Buck, B.H., 2017. Polyculture potential of red Nile tilapia (*Oreochromis niloticus*) and noble crayfish (*Astacus astacus* Linnaeus, 1758) in a recirculating aquaculture system. *Journal of Applied Ichthyology*.
- Seemann, U.B., Lorkowski, K., Slater, M.J., Buchholz, F., Buck, B.H., 2014. Growth performance of Noble crayfish *Astacus astacus* in recirculating aquaculture systems. *Aquaculture International*. 23, 997-1012.
- Seemann, U.B., Lorkowski, K., Slater, M.J., Buck, B.H., Buchholz, F., 2017. Feed alternatives for Noble crayfish *Astacus astacus* based on fatty acid and lipid analyses. *Journal of Shellfish Research*. 36, 519-527.
- Seemann, U.B., Lorkowski, K., Schiffer, M., Hörterer, C., Slater, M.J., Buck, B.H., 2014. Survival of early stripped eggs of the noble crayfish *Astacus astacus* and effects of saline solution during artificial incubation. *Freshwater Crayfish*. 20, 1-6.
- Skurdal, J., Taugbøll, T., 1994. *Biology, Culture and Management of the Noble Crayfish Astacus Astacus L.*
- Skurdal, J., Taugbøll, T., 2001. Crayfish of commercial importance. in: Holdich, D.M. (Ed.), *Biology of Freshwater Crayfish*. Blackwell Science, Oxford, pp. 467-510.
- Stucki P., B., Z., 2011. *Aktionsplan Flusskrebse Schweiz. Artenförderung von Edelkrebse, Dohlenkrebse und Steinkrebse*. Bundesamt für Umwelt, Bern.
- Taugbøll, T., Skurdal, J., 1988. Increased proportion of mature females of the Noble Crayfish (*Astacus astacus* L.) in culture conditions. *Aquaculture*. 69, 39-42.

- Taugbøl, T., Skurdal, J., 1990. Reproduction, Molting and Mortality of Female Noble Crayfish, *Astacus astacus* (L., 1758), From Five Norwegian Populations Subjected To Indoor Culture Conditions (Decapoda, Astacoidea). *Crustaceana*. 58, 113-123.
- Taugbøl, T., Skurdal, J., 1992. Growth, mortality and moulting rate of noble crayfish *Astacus astacus* L., juveniles in aquaculture experiments. *Aquaculture and Fisheries Management*. 23, 411-420.
- Taylor, H.M., Kluger, L.C., 2016. Aqua- and mariculture management – a holistic perspective on best practices. in: Salomon, T.M.a.M. (Ed.), *Marine Environment Protection*. Springer, Heidelberg.
- Telesetsky, A., 2017. Overfishing and Bycatch, *International Farm Animal, Wildlife and Food Safety Law*, pp. 405-431.
- Tilmans, M., Mrugała, A., Svoboda, J., Engelsma, M.Y., Petie, M., Soes, D.M., Nutbeam-Tuffs, S., Oidtmann, B., Roessink, I., Petrusek, A., 2014. Survey of the crayfish plague pathogen presence in the Netherlands reveals a new *Aphanomyces astaci* carrier. *Journal of Invertebrate Pathology*. 120, 74-79.
- Usio, N., Konishi, M., Nakano, S., 2001. Species Displacement Between an Introduced and A 'vulnerable' Crayfish: The Role of Aggressive Interactions and Shelter Competition. *Biological Invasions*. 3, 179-185.
- Vaeßen, S., Groß, H., Nowak, M., 2016. Konzepte zum Schutz des Edelkrebsses vor dem Signalkrebs, forum flusskrebse. *Forum Flusskrebse, Bad Münstereifel*.
- Valipour, A., Ozorio, R.O.A., Shariatmadari, F., Abedian, A., Seyfabadi, J., Zahmatkesh, A., 2012. Effects of dietary lipid levels on growth, survival, and molting of yearling narrow clawed crayfish, *Astacus leptodactylus*. *Journal of Applied Aquaculture*. 24, 316-325.
- Viljamaa-Dirks, S., Heinikainen, S., Nieminen, M., Vennerstrom, P., Pelkonen, S., 2011. Persistent infection by crayfish plague *Aphanomyces astaci* in a noble crayfish population. *Bulletin of the European Association of Fish Pathologists - A case report*. 31, 182-188.
- Vorburger, C., Ribí, G., 1999. Aggression and competition for shelter between a native and an introduced crayfish in Europe. *Freshwater Biology*. 42, 111-119.
- Wagner, R., 2006. *Wasserqualität und Gewässerqualität. Limnologische Fluss-Station Schlitz*.
- Waldmann, B., 2019. *Flusskrebse in Deutschland: Verbreitung – Schutz – Akteure, Fachbereich 3: Mathematik / Naturwissenschaften. Universität Koblenz-Landau, Stuttgart*.
- Walther, M., 2008. *Krebstiere (Crustacea) – Biologie, Vorkommen, Haltung und Erkrankungen, sowie ihre Bedeutung als Zootierobjekte und Lebensmittelressourcen. Eine Literaturstudie., Fachbereich Veterinärmedizin. Justus-Liebig-Universität Gießen, Gießen, pp. 381*.

- Westin, L., Gydemo, R., 1986. Influence of light and temperature on reproduction and moulting frequency of the crayfish, *Astacus astacus* L. *Aquaculture*. 52, 43-50.
- Westman, K., Savolainen, R., 2001. Long term study of competition between two co-occurring crayfish species, the native *Astacus astacus* L. and the introduced *Pacifastacus leniusculus* Dana, in a finnish lake. *Bulletin Français de la Pêche et de la Pisciculture*, 613-627.
- Wickins, J.F.L., Daniel O'C. , 2002. *Crustacean Farming: Ranching and Culture*, 2nd Edition ed. Wiley-Blackwell.
- Wik, T., Lindén, B., 2004. Modeling, Control and Simulation of Recirculating Aquaculture Systems. *IFAC Proceedings Volumes*. 37, 141-146.
- Wittmaack, K.E., 2006. Analyses of nutritional ecology of the Nobel crayfish (*Astacus astacus*) - Diet selection and prey experiments, Zoological Institute - Department Limnology. Christian - Albrechts - University Kiel, Kiel, Germany, pp. 89.
- Xu, W.N., Liu, W.B., Shen, M.F., Li, G.F., Wang, Y., Zhang, W.W., 2013. Effect of different dietary protein and lipid levels on growth performance, body composition of juvenile red swamp crayfish (*Procambarus clarkii*). *Aquaculture International*. 21, 687-697.
- Zimmermann, J.K.M., 2012. Noble Crayfish (*Astacus astacus*) in a Chainging World – Implications for Management, Department of Natural Sciences, Engineering and Mathematics. Mid Sweden University, SE - 851 70 Sundsvall, Sundsvall. Sweden, pp. 39.

Acknowledgements

I am grateful to many people for their long-lasting support and encouragement which enabled the successful completion of this research work. Some of them are gratefully acknowledged here. However, there are many more and to all I want to express a warm and honest: Thank you.

First, I would like to thank Prof. Dr. Bela H. Buck, Prof. Dr. Friedrich Buchholz and Dr. Matthew J. Slater who provided great scientific support to finish this work. Thank you, Bela for letting me write the proposal, chose this topic and for your understanding and your guidance though the last years. Matt for giving me the space in your group to work as I wanted. I appreciate that. Big thanks for your support. Furthermore, I would like to thank all group members, especially the ZAF team for their logistic and creative assistance: Kai Lorkowski, Gregor Jähne, Rajko Thiele and Nina Kröncke. A warm thank you to my project partners Michaela and Steffen Göckemeyer and to Kathrin Theissing and Anne Schrimpf from the University Koblenz-Landau.

For the ungrateful task of proofreading special thanks go to Matthew and Bela for their thorough work, for happy discussions and many, many helpful comments.

Without the funding of the DBU this work would not have been possible so grateful thanks to Osnabrück and especially to Dr. H. Wurl.

Finally, I would like to thank my family for everything and myself for bringing this to an end! Thank you, Tina, Johan, Oskar, "little one" on your way to us and Mum and Dad for being there!

Declaration on the contribution of the candidate to multi-author articles which are included as chapters in the submitted doctoral thesis

Chapter I: Growth performance of Noble crayfish *Astacus astacus* in recirculating aquaculture systems.

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. 95%
Experimental work and/or acquisition of (experimental) data:	ca. 90%
Data analysis and interpretation:	ca. 80%
Preparation of Figures and Tables:	ca. 50%
Drafting of the manuscript:	ca. 95%

Chapter II: Feed alternatives for Noble crayfish *Astacus astacus* based on fatty acid and lipid analyses!

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. 90%
Experimental work and/or acquisition of (experimental) data:	ca. 65%
Data analysis and interpretation:	ca. 75%
Preparation of Figures and Tables:	ca. 75%
Drafting of the manuscript:	ca. 95%

Chapter III: Survival of early stripped eggs of the noble crayfish *Astacus astacus* and effects of saline solution during artificial incubation

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. 85%
Experimental work and/or acquisition of (experimental) data:	ca. 70%

Data analysis and interpretation:	ca. 75%
Preparation of Figures and Tables:	ca. 65%
Drafting of the manuscript:	ca. 95%

Chapter IV: Polyculture potential of red Nile tilapia (*Oreochromis niloticus*) and noble crayfish (*Astacus astacus* Linnaeus, 1758) in a recirculating aquaculture system

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. 90%
Experimental work and/or acquisition of (experimental) data:	ca. 20%
Data analysis and interpretation:	ca. 80%
Preparation of Figures and Tables:	ca. 70%
Drafting of the manuscript:	ca. 95%

Chapter V: Shelter preference of Noble crayfish *Astacus astacus* in recirculating aquaculture systems

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. 95%
Experimental work and/or acquisition of (experimental) data:	ca. 40%
Data analysis and interpretation:	ca. 90%
Preparation of Figures and Tables:	ca. 90%
Drafting of the manuscript:	ca. 95%

Date: 02.09.2019

Signatures: 

Erklärung

Hiermit erkläre ich, dass ich die Doktorarbeit mit dem Titel:

Nachhaltige Besatzkrebiszucht in einem geschlossenen Aquakultur-Kreislaufsystem am Beispiel des bedrohten Edelkrebse*s Astacus astacus*

selbstständig verfasst und geschrieben habe und außer den angegebenen Quellen keine weiteren Hilfsmittel verwendet habe.

Ebenfalls erkläre ich hiermit, dass es sich bei den von mir abgegebenen Arbeiten um drei identische Exemplare handelt.

Sellstedt, August 2019

U. Seemann