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Growth performance and RNA/DNA ratio of noble crayfish (*Astacus astacus*) and narrow-clawed crayfish (*Pontastacus leptodactylus*) fed fish waste diets

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Abstract

New and viable species for aquaponics and integrated multi-trophic aquaculture (IMTA) in freshwater systems can improve yields and sustainability of aquaculture. Freshwater crayfish species such as *Astacus astacus* and *Pontastacus leptodactylus* are omnivorous feeders and considered candidates for feeding on faecal matters in existing aquaculture systems. Feeding trials were conducted to determine growth response and RNA/DNA ratio in freshwater crayfish fed fish waste. Carapace length and wet weight were measured to determine the growth response. Juvenile *A. astacus* was fed faeces of hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) and rainbow trout (*Oncorhynchus mykiss*), while adult *P. leptodactylus* was fed with two commercial pellet diets and pikeperch (*Sander lucioperca*) faeces. The nutritional composition of hybrid striped bass faeces was close to optimal diet composition of *A. astacus*, and crayfish showed significantly higher carapace growth, weight gain and weight gain per moult as the group fed rainbow trout faeces. The growth of *P. leptodactylus* was significantly lower in terms of weight gain and weight gain percentage per moult for crayfish fed on pikeperch faeces. Thus, this study can recommend a co-cultivation of hybrid striped bass and *A. astacus* within one system, but cannot recommend co-cultivation of *P. leptodactylus* with pikeperch. Additionally, this study showed controversial results of RNA/DNA ratio and weight gain of both crayfish species. Thus, RNA/DNA ratio cannot be approved for investigations on crayfish physiological status in controlled feeding experiments if animals are fed with an inadequate diet.

KEYWORDS
freshwater crayfish, growth performance, IMTA, integrated aquaponics, RNA/DNA ratio

1 | INTRODUCTION

Increasing energy costs and limited availability of natural resources drive demand to improve aquaculture system efficiency. Fish species still dominate aquaculture production and value but feed conversion ratio (FCR) values remain poor for many fed species and faeces are correspondingly nutrient-rich (Davenport et al., 2003; MacDonald, Stead, & Slater, 2013; Sugiura, Dong, Rathbone, & Hardy, 1998).

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Aquaponics and integrated multi-trophic aquaculture systems (IMTA), where wastes including faeces are consumed by lower trophic levels, are considered models of sustainable integrated food production with future potential (Chopin, Cooper, Reid, & Cross, 2012; Troell et al., 2003). Crayfish species are low trophic level feeders of increasing importance in aquaculture due to excessive exploitation of natural stocks and growing demand on the national and international market (Ackefors, 2000; Ackefors, Castell, Boston, Räty, & Svensson, 1992; Franke, Wessels, & Hörstgen-Schwark, 2013; Holdich, 2002; Seemann, Lorkowski, Slater, Buchholz, & Buck, 2015). As omnivores and detritivores (Olsson, 2005, 2008), crayfish can be considered promising candidates for aquaponics systems or IMTA if they are suited to the physicochemical parameters prevailing in the aquaculture system. But there are still problems with the assessment of the physiological condition of crayfish individuals, which is measured by highly variable body growth or weight gain. Intermittent growth because of moulting periods and varying frequency of moults (Aiken & Waddy, 1992; Hartnoll, 1982) leads to long observation periods with increasing crayfish age. Alternative biochemical indicators like ribonucleic acid (RNA)/deoxyribonucleic acid (DNA) ratio, which react faster to varying environmental conditions, may offer an essential tool to more accurately determine changes in physiological status over shorter timeframes and increase the efficiency of crayfish cultivation together with shortening experimental times.

Use of RNA/DNA ratio is described as a less time-consuming method for measurements of invertebrate physiological condition and the identification of nutritional status (Grimm, Lehmann, & Clemmesen, 2015; Koop, Winkelmann, Becker, Hellmann, & Ortmann, 2011; Wolf, 2004). The ratio is composed of the constant quantity of DNA and a varying quantity of RNA in cells. While DNA content is not affected by cell processes, RNA content varies by protein synthesis rate. Consequently, the protein synthesis rate can be well determined by measuring the RNA/DNA ratio (Buckley, 1979; Henshaw, Hirsch, & Morton, 1971). Previous studies on crustaceans showed precise results from RNA/DNA analyses for the effect of different feeding intervals (Grimm et al., 2015; Moss, Harbor, & Loa, 1994), different algal diets (Moss, 1994) and even within different developmental stages (Moss, 1994; Moss et al., 1994; Vrede, Persson, & Aronsen, 2002).

Therefore, this method was included in the current study as an attempt to determine the physiological status of *Astacus astacus* and *Pontastacus leptodactylus* feeding on different commercial diets and fish faeces, respectively, with the longer-term intention to gain insight into the feasibility of rearing them in aquaponics or IMTA. These two species were chosen specifically to match the temperature regimes of the finfish species (and corresponding faeces diets) with which they would ostensibly be grown in IMTA. In accordance with the literature, a correlation between somatic growth performance, total length and carapace growth as well as weight gain, and RNA/DNA ratio can be expected. Furthermore, RNA/DNA ratio should react more sensitively to nutritional and therefore physiological status within crayfish and detect differences, which cannot yet be seen in growth performance.

2 | MATERIAL AND METHODS

2.1 | Animals and diets

Two different crayfish species, *A. astacus* and *P. leptodactylus*, were investigated in paired experiments. *Astacus astacus* were obtained from 'Krebszucht Oeversee' (H. Jeske, Oeversee, Germany) and *P. leptodactylus* from 'Edelkrebse.online' (Burbach, Germany). Both species were grown in ponds under ambient conditions. Animals were fished onsite, and a representative number of animals reflecting the size and sex mix of the aquaculture systems were selected. Prior to the experiment, all animals were acclimated under controlled conditions in the experimental tanks at the Centre for Aquaculture Research of the Alfred Wegener Institute, Bremerhaven, Germany, for at least five weeks. *Astacus astacus* were fed with 'Aller Metabolica' pelleted diet (Emsland Aller Aqua GmbH) and *P. leptodactylus* with 'LeGouessant B-Peneaus-Grower RCE 3′ pelleted diet (Le Gouessant Aquaculture) once every afternoon except on the weekends (Table 1).

Before starting each experiment, wet weight (WW) of all crayfish was individually determined (TE412 balance; Sartorius, ±0.01 mm) and carapace length (CL) was measured using a digital calliper (Hoffman Group). One hundred twenty *A. astacus* with a mean WW of 2.05 ± 0.03 g and a mean CL of 20.3 ± 0.1 mm were randomly allocated in groups of ten into twelve 200-L tanks. A total of 99 *P. leptodactylus* with a mean WW of 30.1 ± 8.2 g and a mean CL of 51.4 ± 4.5 mm were treated as well and stocked in groups of 11 crayfish in nine 200-L tanks.

2.2 | Experimental design and management of *Astacus astacus* growth trial

Two different types of fish faeces were provided to two treatment groups of *A. astacus*, each with six replicates and ten animals (22.5 crayfish/m^2) per replicate (Figure 1, Table 2). Fifteen animals were initially sampled, before dividing the tanks into the two feeding groups to represent t_0 for later RNA/DNA analysis. The two feeding regimes were maintained exclusively with faeces produced by rainbow trout (*Oncorhynchus mykiss*) or hybrid striped bass (*Morone chrysops* × *M. saxatilis*). Fish were fed with a commercial diet in the morning and the afternoon, totalling a daily feeding rate of 1% body

TABLE 1 Ingredients and proximate composition of the commercial diets

	LeGouessant B-Peneaus-Grower RCE 3	Aller Metabolica (3 mm)
Crude protein (%)	38	52
Crude fat (%)	8	15
Ash (%)	13	7
Fibre (%)	1.1	2
P in dry matter (%)	1.8	1.2

weight. The crayfish were continuously supplied with fish faeces from the fish tanks, which were connected by water flow (see arrows Figure 1). The recirculating system was provided with a cooling unit to meet the water temperature requirement of rainbow trout of 16.40 ± 0.07°C and water parameters recorded. After eight weeks, this experiment was terminated, and all crayfish were frozen in liquid nitrogen for further RNA/DNA quantification. Water quality parameters stayed within reported tolerance ranges of *A. astacus* throughout the experiment with the exception of nitrate (Table 3).

2.3 | Experimental design and management of *Pontastacus leptodactylus* growth trial

Three different diet treatments were provided to *P. leptodactylus* in the controlled feeding experiment: LeGouessant B-Peneaus-Grower RCE 3 (control group), Aller Metabolica (Table 1) and faeces of pikeperch (*Sander lucioperca*; Table 2). As there is no commercial formulated diet specifically for *P. leptodactylus*, the experimental diets were selected considering the lipid and protein requirements of the crayfish and their commercial availability. During the acclimation phase, animals were kept in nine tanks with 11 animals each. Prior to experimental onset, three crayfish from each acclimation tank were randomly sampled and frozen in liquid nitrogen to represent t_0 for later RNA/DNA analysis. Subsequently, three replicate tanks were each stocked with eight animals each (17.8 crayfish/m^2), represented control group (CG; LeGouessant B-Peneaus-Grower RCE 3), fish food group (AM; Aller Metabolica) and faeces group (PF; pikeperch faeces; Figure 2). Faeces were sourced from a nearby pikeperch farm and dried at 120°C overnight. All tanks were fed with 1.5% of the overall biomass in the specific tank every afternoon except on weekends. To regularly adjust the feeding amount, crayfish growth performance was measured every two weeks. The recirculating system was provided with a cooling unit to meet the water temperature requirement of the *P. leptodactylus* (and pikeperch) of 23.4 ± 0.5°C and water parameters recorded. After ten weeks, this experiment was terminated, and all crayfish frozen in liquid nitrogen for later RNA/DNA analysis. During the experiment phase, crayfish were maintained in a flow-through system with 12:12-hr light:dark photoperiod, continuous aeration and monitored water parameters (Table 1).

2.4 | RNA/DNA quantification

To determine RNA and DNA concentration, 50–55mg of muscle samples was taken from the abdomen of frozen animals and stored in −80°C before analysis. Muscle samples were extracted on an ice-cooled glass plate to prevent thawing of the tissue. The muscle samples were analysed by Q–Bioanalytic GmbH in Bremerhaven. As a first step, 1ml CTAB lysis buffer and 10µl proteinase K were placed in a 1.5-ml Eppendorf tube. Subsequently, 200mg of sample material was added, and the mixture was shaken for at least 2hr at 60°C. An extraction control (reagents without sample material) was also analysed per 10 samples. Afterwards, 10µl RNase was added. Samples were incubated for 2min at room temperature and centrifuged for 2min at 10,000 *g* 900µl of the resulting supernatant was added to 600µl chloroform (phenol mixture), which was previously filled to the reaction tube. This was followed by vortexing and 10 min centrifuging at 10,000 *g*. 625µl of the supernatant was taken and added to 500µl isopropyl and 2µl of glycogen. This mixture was mixed by inverting. The precipitation of the DNA took place overnight. The next day, the samples were centrifuged for 10min at 10,000 *g*, and the resulting supernatant was discarded. 500µl of 75% ethanol was added and vortexed, to detach the pellet. This mixture was again centrifuged for 5min at 10,000 *g*, and the resulting supernatant was carefully discarded with the help of a pipette. The

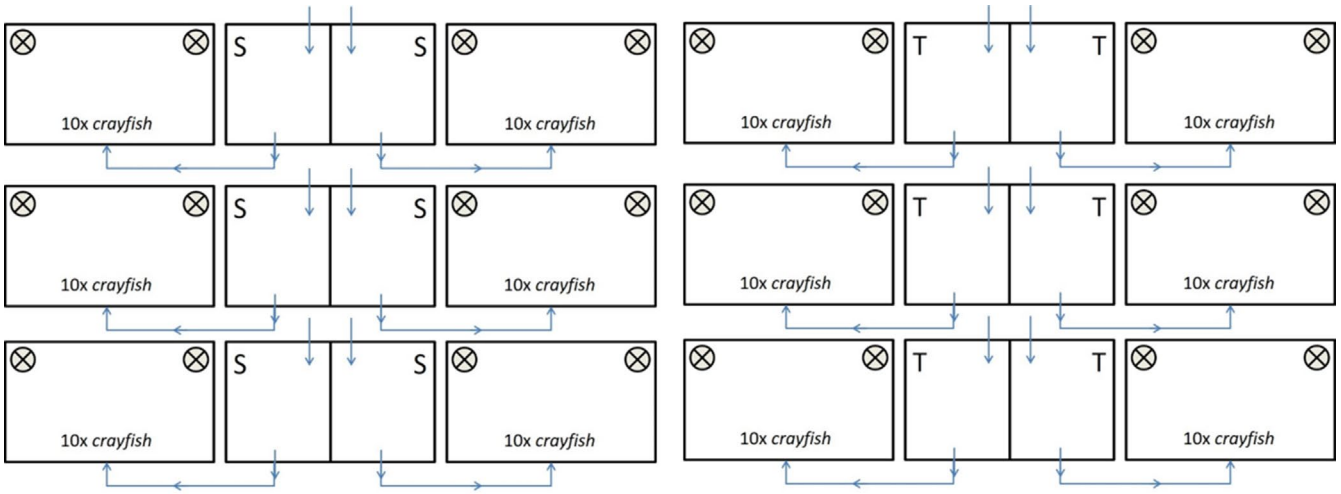

FIGURE 1 A total of 18 tanks (200L, 0.44m^2) were used for *Astacus astacus*. Crayfish were held in the original tank size (200L), and fish were kept in the six internal tanks (100L). The arrows show the water flow in the system (from the water inlet in the fish tanks (S: tanks contain hybrid striped bass, T: tanks contain rainbow trout) through the pipe connection into the crayfish tanks). Crayfish tanks include surface outflows ⊗ to allow the faeces to settle [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Proximate composition of hybrid striped bass (S), rainbow trout (T) and pikeperch (PF) faeces.

	Moisture (%)	Nutrient composition (g 100g^{-1} dry matter)				Gross energy (MJ/kg)
		Crude protein	Crude lipid	Crude ash	NFE	
Astacus astacus						
S	3.4	40.9	9.4	20.7	29.0	28.8
T	4.0	28.7	11.9	26.0	33.4	17.9
Pontastacus leptodactylus						
PF	4.8	37.1	11.2	9.4	46.9	/

TABLE 3 Mean water parameters during the experiment with *Astacus astacus* and *Pontastacus leptodactylus*

	A. astacus (Mean ± σ)	*P. leptodactylus* (Mean ± σ)
O_2–saturation [%]	96.722 ± 0.295	99.038 ± 1.041
Temperature [°C]	16.402 ± 0.073	23.409 ± 0.505
pH	7.135 ± 0.042	8.324 ± 0.205
NH_4^+ - N [mg/L]	0.045 ± 0.008	0.003 ± 0.006
NO_2^- - N [mg/L]	0.037 ± 0.003	0.031 ± 0.016
NO_3^- - N [mg/L]	36.960 ± 0.905	8.939 ± 1.809

pellet was optionally dry at RT. After drying the pellet, it was dissolved with 50–100µl of 0.2 TE buffer. The purification was carried out in duplicate, and all steps were carried out on ice. The fluorescence of the samples and standards was measured using the DNA program NanoDrop 2000. The photometric measurements revealed results for measurements before RNase treatment and measurements after RNase treatment (DNA concentration). RNA/DNA ratio was calculated as follows:

$$\frac{\text{RNA}}{\text{DNA}}\text{ratio} = \frac{(\text{A sample} - \text{B sample})}{\text{B sample}}$$

where A sample is the nuclei acid concentration before RNase treatment, and B sample is the nuclei acid concentration after to RNase treatment.

2.5 | Chemical analyses

Proximate analyses have been performed in duplicate determination and according to the Regulation (EU) No 1169/2011 (The European Parliament and of the Council, 25th of Oct 2011). The determination of dry matter (DM, drying at 103 ± 2°C for four hours), crude protein (Kjeldahl, N × 6.25), crude lipid (petroleum extraction and Soxhlet method) and ash (combusting at 550°C) was carried out in the laboratory facilities of the University of Applied Sciences (Bremerhaven, Germany). Gross energy was determined by an automated oxygen bomb calorimeter (Parr 6100 calorimeter; Parr Instrument GmbH)

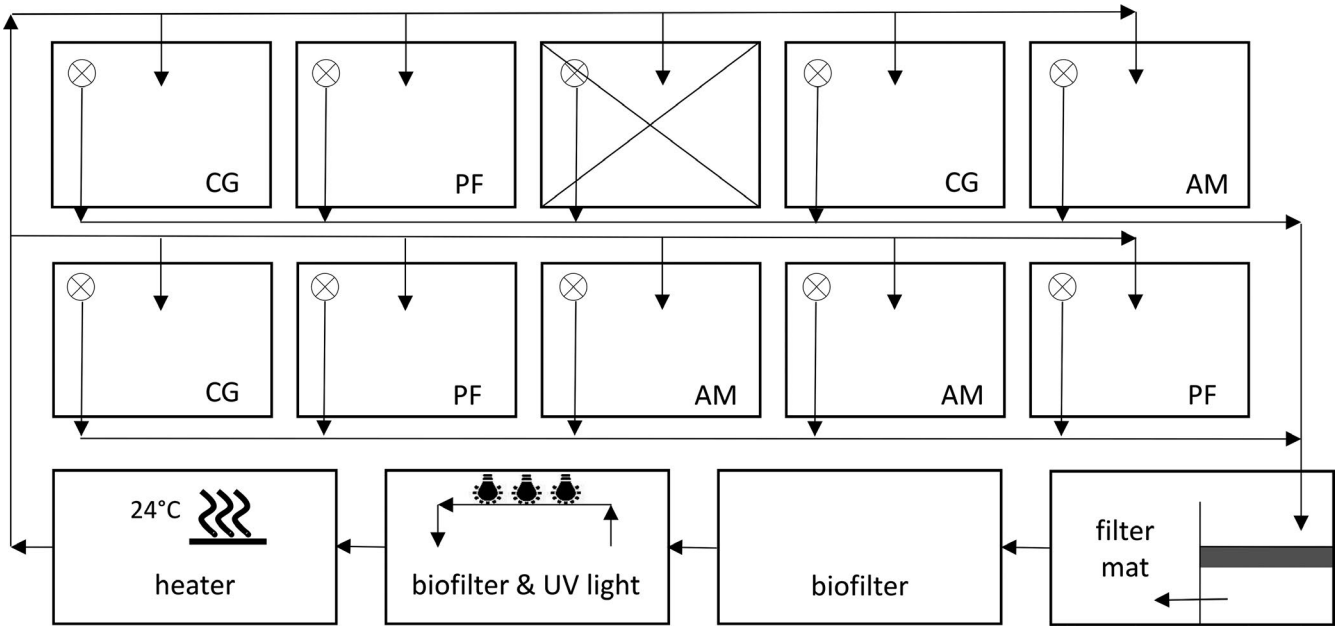

FIGURE 2 A total of nine tanks (200L, 0.45m^2) were used for *Pontastacus leptodactylus*. The arrows show the water flow in the system and crayfish tanks. All crayfish tanks include surface outflows ⊗

at the Alfred-Wegener-Institut (AWI) facilities. Nitrogen-free extract (NFE) was calculated by subtraction (DM minus crude protein, crude lipid and crude ash).

2.6 | Evaluation of growth response

Biomass and carapace length of each animal were measured fortnightly during the whole experiment. Mortality was checked daily, and dead animals were removed. Also, exuvia were removed from the tanks as soon as they were observed to avoid the animals to feed on them.

Performance calculations were as follows:

$$\text{Moult rate}\,(\%) = \frac{100 \times \text{number of moulted animals}}{\text{initial number}}$$

$$\text{Death rate}\,(\%) = \frac{100 \times \text{final number}}{\text{initial number}}$$

$$\text{Carapace increment}\,(\text{cm}) = \text{final}_{\text{carapace length}} - \text{initial}_{\text{carapace length}}$$

$$\text{Weight gain}\,(\text{g}) = \text{final}_{\text{WW}} - \text{initial}_{\text{WW}}$$

$$\text{Mean weight gain per moult}\,(\%) = \left(\text{WW}_{\text{post moult}} - \text{WW}_{\text{pre moult}}\right) \times \frac{\text{WW}_{\text{pre moult}}}{100}$$

2.7 | Data analysis

Data analysis was conducted using R 3.5.1 using RStudio. Data were tested for normality using a Shapiro–Wilk test and homoscedasticity with a Levene's test. Where assumptions were fulfilled, an ANOVA and Tukey's post hoc test was used to determine differences between growth performance and RNA/DNA ratios of the tested diets. For non-normal or heteroscedastic data, a Kruskal–Wallis test with a Wilcoxon Mann–Whitney post hoc test was used. All statistical tests were considered significant at $p < .05$.

2.8 | Ethics statement

Both experiments were registered with the Veterinary Authority of the State of Bremen, Germany, under the category—registerable experiments with decapods. All efforts were made to ensure no suffering of experimental animals and to ensure that the appropriate number of animals was tested to provide robust results but a minimum of animal loss.

3 | RESULTS

3.1 | Nutrient analysis of faeces

The results of the nutrient analysis of faeces showed that hybrid striped bass faeces were 30% richer in protein and 15% in energy as trout faeces (Table 2). However, trout faeces had a 20% higher lipid level account to 11.9 g 100 g^{-1} DM. Pikeperch faeces contained markedly lower crude ash (9.4%) when compared to all other faeces types. Pikeperch faeces had a similar amount of protein (37.1%) to seabass faeces and a very similar lipid content to trout faeces.

3.2 | Astacus astacus

Faeces tested did not affect moulting ($p = .752$) and death rate ($p = .8693$) of *A. astacus* significantly. Carapace increment was significantly higher ($p = .0313$) in the group fed with hybrid striped bass faeces (S) as in the group fed with rainbow trout faeces (T) (Figure 3). Weight gain was significantly higher in the S treatment ($p = .002$), as was percentage weight gain per moult ($p = .049$). Mean percentage weight gain in the S treatment was 28.49 ± 19.46 (max: 124.84% and min: 0%) and 22.34 ± 14.08 within group T (max: 51.37% and min: 0%). RNA/DNA ratio also differed significantly between S and T ($p = .005$) and between t_0 and S. Unlike carapace increment and weight gain, the RNA/DNA ratio is higher in T than in S (Figure 3).

3.3 | Pontastacus leptodactylus

Moulting ($p = .107$) and death rate ($p = .236$) did not differ significantly between the three diet treatments for *P. leptodactylus*. Nevertheless, the highest moulting rate of 37.5% was detected in the treatment fed with Aller Metabolica (AM) and lowest (16.7%) in the treatment fed with pikeperch faeces (PF). Furthermore, PF showed highest death rate with 25% and the control group (CG) showed the lowest death rate (4.2%). Mean carapace increments and mean total length growth did not differ significantly between treatments ($p = .971$ and .566 respectively). Weight gain was significantly higher in AM than PF ($p = .026$ AM-PF). This difference was markedly more obvious within the female crayfish ($p = .001$). In addition to the difference between females of the groups AM and PF ($p = .001$), there was a significant difference between females in the groups CG and PF ($p = .019$). In contrast, there was no significant difference between the three groups within the males ($p = .852$). Nevertheless, mean weight gain was highest in CG within the males and mean weight gain was best in AM within the females (Figure 4). There was no significant difference observed between the three groups divided into males and females in carapace increment or length growth. In contrast to overall weight gain results, percentage weight gain per moult was significantly higher in CG than in AM ($p = .041$) and PF ($p = .045$; Figure 4). RNA/DNA ratio did not differ significantly ($p = .803$) within the treatments or between the beginning of the experiment and the different treatments (Figure 4). There was also no significant difference between the groups or the beginning and the end of the experiment dividing crayfish into males ($p = .967$) and females ($p = .744$; Figure 5).

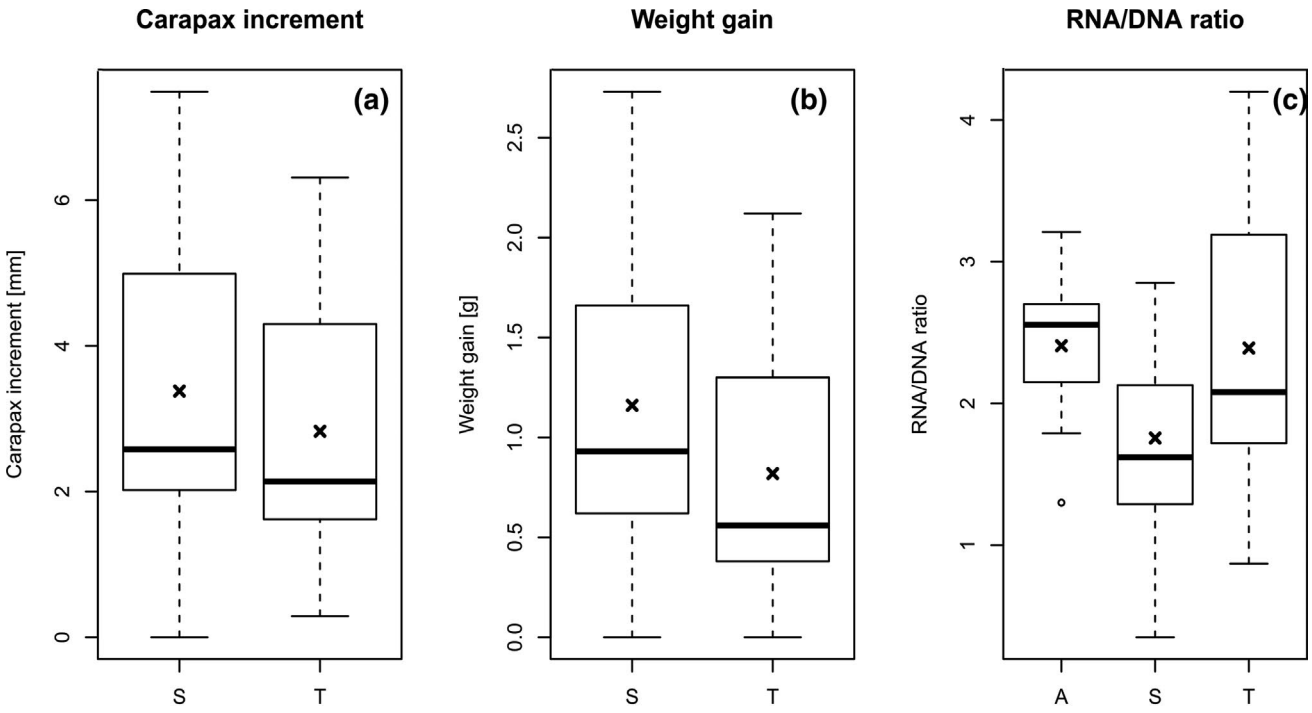

FIGURE 3 Growth performance of *Astacus astacus* divided into carapace increment (a) and weight gain (b) within the two treatments (S, T) and RNA/DNA ratio of *A. astacus* (c) at the beginning of the experiment t_0 ('start') and within the two feeding groups (S, T). The × marks the mean value of each treatment (carapace increment and weight gain: N(S) = 58, N(T) = 57; RNA/DNA ratio: N(t_0) = 10, N(S) = N(T) = 29)

4 | DISCUSSION

4.1 | Dietary macronutrients

Macronutrients such as protein, lipids and carbohydrate provide energy and essential requirements for growth, maintenance, reproduction and activity (Davis, 2015). For good growth performance and health, nutrients must be provided to meet the requirements of the cultured animal and to create a balance between energy consumption and intake. If energy intake exceeds expenditure or expenditure decreases, the animal can gain in biomass. Conversely, weight loss occurs when the energy input is less than the expenditure (Wolf, 2004). Nutrient requirements of freshwater crayfish are not fully elucidated, but many experiments have been conducted to try to understand the nutritional needs of species of interest. Results of Ackefors et al. (1992) and Seemann et al. (2015) showed that *A. astacus* fed with diets consisting of 30%–40% protein content had the highest mean biomass increase. In addition to dietary protein content, lipid content also has a significant effect on growth. Freshwater crayfish require low levels of lipids in their diets (D'Abramo & Conklin, 1985; Fotedar, 2004), and there are indications of growth inhibition when the levels exceed 8% of total nutrient content (D'Abramo, 1979). Thus, Seemann et al. (2015) recommended culturing *A. astacus* in a recirculating aquaculture system with a diet lipid content of <13%. Results of the present study correspond to these recommendations. *Astacus astacus* fed with hybrid striped bass faeces (protein content 40.9%, lipid content 9.4%) showed better growth performance as *A. astacus* fed with rainbow trout faeces (protein content 28.7%, lipid content 11.9%). Protein content, as well as lipid content of rainbow trout faeces, was unfavourable.

There are numerous studies on the requirements of crude protein and fat for *P. leptodactylus* (Ackefors et al., 1992; Carral et al., 2011; Ghiasvand, Matinfar, Valipour, Soltani, & Kamali, 2012; Valipour, Shariatmadari, Abedian, Seyfabadi, & Zahmatkesh, 2011). Ghiasvand et al. (2012) recommend a protein level between 30% and 39%. Diets containing higher amounts of protein would be more expensive and have no positive effect on growth. Valipour et al. (2012) described lower growth performance and lower survival with lipid contents of 4% and 7% and best growth performance at 10%–13% diet lipid content. In this study, *P. leptodactylus* fed with shrimp feed (CG: protein content 38% and lipid content 8%) and pikeperch feed (AM: protein content 52% and lipid content 15%) showed similar growth performance. Nevertheless, both diets were unfavourable because the CG diet contained a lower amount of lipids and AM diet contained excessively high protein levels and higher lipid content than recommended. Within other species like *Penaeus monodon* (Alava & Lim, 1983) or *Scylla serrata* (Catacutan, 2002) at a specific level, rising protein content had adverse effects on growth performance. Similar results could be observed for lipid level for *Procambarus acutus acutus* (Davis & Robinson, 1986) and *Procambarus clarkii* (Jover, Fernández-Carmona, Del Río, & Soler, 1999). Not only protein content but protein source can be critical in diet formulation. Safari, Shahsavani, Paolucci, and Mehraban Sang Atash (2014) showed that digestibility of protein sources varies highly for *P. leptodactylus* with high protein plant sources and animal protein sources significantly better digested. The digestibility of protein from fishmeal and soy

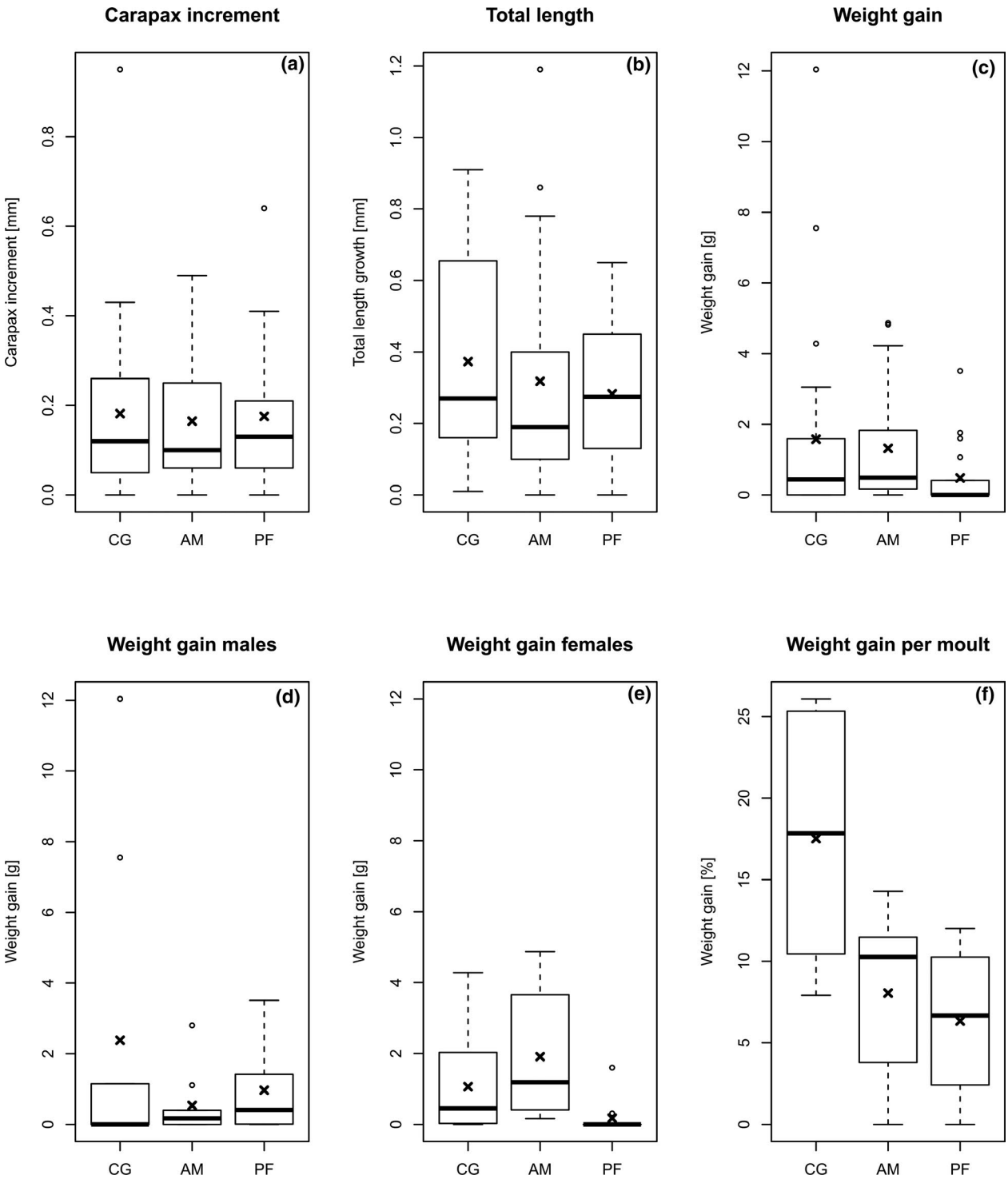

FIGURE 4 Growth performance of *Pontastacus leptodactylus* divided into carapace increment (a), total length growth (b) and weight gain (c) within the three treatments (N(CG) = 23, N(AM) = 21, N(PF) = 18) and weight gain of *P. leptodactylus* divided into males (d; N(CG) = 9, N(AM) = 9, N(PF) = 7) and females (e; N(CG) = 14, N(AM) = 12, N(PF) = 11) and percentage weight gain per moult (f; N(CG) = 5, N(AM) = 9, N(PF) = 4) within the three treatments. The × marks the mean value of each treatment

sources in the faeces diets used in the current study is difficult to estimate after diet processing through the fish gut. However, overall protein digestibility is very likely to be lower in the faeces diets than those tested in previous studies (Safari et al.., 2014). Pikeperch faeces protein and lipid levels fit within the recommended margin with a protein content of 37.1% and lipid content of 11.2%. Furthermore, freshwater crayfish are considered to be omnivorous species and consume detritus (Olsson, 2005, 2008), which can result in similar

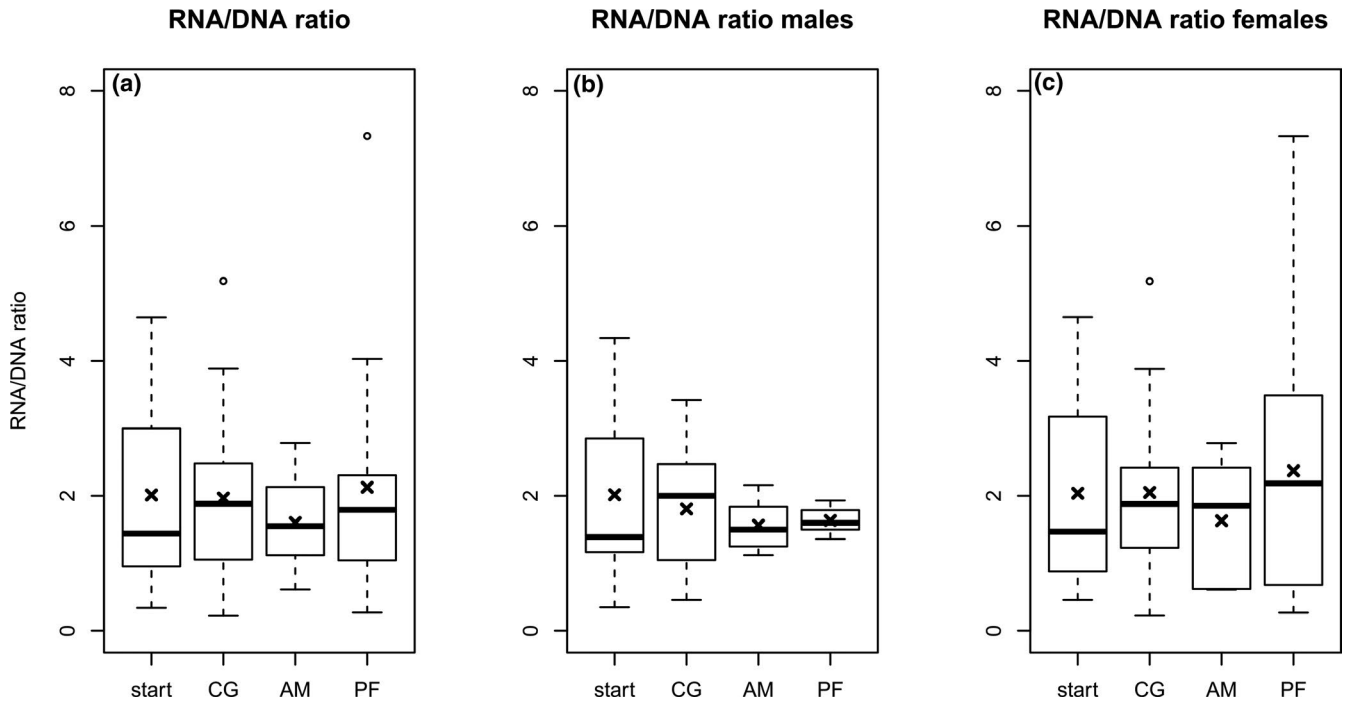

FIGURE 5 RNA/DNA ratio of *Pontastacus leptodactylus* at the beginning of the experiment t_0 ('start') and within the three treatments at the end of the experiment. The × marks the mean value of each treatment (RNA/DNA ratio: N(t_0) = 27, N(CG) = 20, N(AM) = 17, N(PF) = 15; RNA/DNA ratio males: N(t_0) = 7, N(CG) = 9, N(AM) = 7, N(PF) = 5; RNA/DNA ratio females: N(t_0) = 20, N(CG) = 11, N(AM) = 10, N(PF) = 11)

growth performance with pikeperch faeces and CG and AM diets. Nevertheless, Momot (2008) argued that crayfish do not consume as much detritus as expected, and the role of crayfish in the ecosystem must be redefined. Therefore, unfavourable macronutrient levels of CG and AM diets could also explain the lack of distinct difference in length and carapace growth between the different diets.

4.2 | Growth performance

In the current study, growth performance did not differ significantly except weight gain between CG, AM and PF. Generally, the growth of crayfish depends on moulting, which is influenced by food supplies, photoperiod, water quality and nutrition levels (Aiken & Waddy, 1992; Lucas & Southgate, 2012). Since water quality parameters and photoperiod were equal in each tank for both growth trials, the different moult frequencies might be due to the various food supplies and the corresponding nutrition levels of the crayfish. Expected weight gain per moult for crayfish according to Aiken and Waddy (1992) was about 30%–60% per moult, but in this study, a maximum mean weight gain percentages of 28.5% (*A. astacus* group S) and 17.5% (*P. leptodactylus* group CG) were achieved. Previous studies by Safari et al. (2014); Safari, Shahsavani, Paolucci, and Mehraban Sang Atash (2015); Seemann et al. (2015); Valipour, Nedaei, Noori, Khanipour, and Hoseinifar (2019) have indicated that variable growth and body size in *P. leptodactylus* and *A. astacus* can result from a suboptimal nutrient provision in diets. The variable growth and suboptimal survival rates observed in the current study indicate that faeces diets alone are not capable of providing an acceptable nutrient density and diversity to the species investigated.

Lower weight gains recorded for *A. astacus* may be explained by the lack of an appropriate diet and/or non-optimal holding temperatures. The recommended water temperature to culture *A. astacus* is between 18 and 21°C, with a minimum of 15°C and a maximum of 25°C (Arzbach, 2010; Cukerzis, 1988; Hager, 2003). However, the optimum water temperature to grow rainbow trout is between 12 and 18°C (Purser & Forteath, 2012), and to fulfil this requirement, water temperature of this experiment was set to 16°C. For this reason, the water temperature was on the lowest limit of *A. astacus* not providing optimal growth conditions. In contrast, *P. leptodactylus* were within their optimal environmental conditions range, but all diets seemed to be inappropriate. CG and AM diets do not fit the optimum macronutrient requirements, and pikeperch faeces remain untried as crayfish diet to date.

4.3 | RNA/DNA ratio

There are a few studies on the RNA/DNA ratio relating to crayfish, and this study reveals the effect of cultivation of crayfish feeding exclusively on faeces for the first time. Grimm et al. (2015) compared measured growth parameters such as carapace length, wet weight and specific growth rates (SGR) with RNA/DNA ratios for juvenile *A. astacus*, which were fed with frozen *Daphnia* sp. After five-week experiment duration, crayfish had a RNA/DNA ratio of 1.23 ± 0.08 (±*SD*) with a final wet weight of 88.1 ± 24.3 mg and

a CL of 8.2 ± 0.6 mm. Higher RNA/DNA ratios were found in the study of Wolf (2004) for the signal crayfish (*Pacifiastacus leniusculus*), which were fed at different feeding intervals. Crayfish with a final CL of 30.04–31.32 mm and a final wet weight between 7.73 and 9.10 g showed RNA/DNA ratios from 6.64 to 8.89. RNA/DNA ratio of the present study was similar to the results of Grimm et al. (2015) and ranged between 2.41 ± 0.54 (t_0), 1.76 ± 0.59 (S) and 2.39 ± 0.89 (T) for *A. astacus* and between 2.03 ± 1.3 (t_0), 1.94 ± 1.2 (CG), 1.61 ± 0.69 (AM) and 2.13 ± 1.72 (PF) for *P. leptodactylus*.

High accuracy of RNA/DNA ratios compared to carapace length, wet weight and SGR was observed by Grimm et al. (2015); a high growth performance led to higher RNA/DNA ratios. In the present study, the inverse correlation (*A. astacus*) and no correlation (*P. leptodactylus*) of RNA/DNA ratios and growth performance were observed. None of the significant growth performance differences monitored in this study was reflected in the RNA/DNA ratio. According to previous studies on RNA/DNA ratio, similar values indicate similar physical status, which does not coincide with growth performance results of the present study. Wolf (2004) examined the effect of different binding agents on the growth performance of signal crayfish and reported uncorrelated results for growth performance and RNA/DNA ratio as well. These uncorrelated results were explained by the higher energy input the crayfish require for their digestion leading to the assumption that the high RNA/DNA ratios did not result from the protein synthesis used for tissue growth, but from digestive metabolism. Wang et al. (2006) described different digestive enzyme activity for *Pelteobragus fulvidraco* fed diets differing only in protein concentrations. Varying enzyme activity patterns were also described for copepods (Jones, Kumlu, Le Vay, & Fletcher, 1997), *Hyas araneus* (Harms, Anger, Klaus, & Seeger, 1991) and artemia (Samain, Moal, Daniel, Coz, & Jezequel, 1980) according to diet. Rodriguez, Le Vay, Mourente, and Jones (1994) reported six times higher activity of the digesting enzyme trypsin for *Penaeus japonicas* (Bate) feeding on a herbivorous diet than with a carnivorous or mixed diet. Consequently, non-correlating RNA/DNA ratios and growth performance data can result from different metabolic activities required for digestion of different diets.

5 | CONCLUSION

This study addressed two questions: whether the two crayfish species are promising candidates for IMTA and whether the RNA/DNA ratio can be used as an early parameter for physiological status if no suitable feed is supplied.

In terms of crayfish inclusion into an IMTA, it must be considered that in this approach, the crayfish is cultivated as a by-product and therefore a lower growth performance is not an exclusion criterion for the integration. The faeces content of hybrid striped was close to the diet requirements of *A. astacus*, and crayfish showed a significantly higher growth performance as the group fed with rainbow trout faeces. In contrast, the growth performance of *P. leptodactylus* was significantly lower for crayfish fed on pikeperch faeces. Thus, this study can recommend a co-cultivation of hybrid striped bass and *A. astacus* within one system, if the crayfish, even in long-term trials, show continuous growth with low mortality rates. *Pontastacus leptodactylus* cannot be recommended for co-cultivation with pikeperch.

Due to the conflicting results, RNA/DNA ratio analysis did not show any helpful results for a clear understanding of the crayfish growth, when feeding crayfish, different types of diet or fish faeces. High RNA/DNA ratios resulted from a high RNA concentration and can be explained by the fact that RNA concentrations assumed to indicate growth can be obscured by other physiological processes such as moulting and digestion. Consequently, the present study demonstrates that the RNA/DNA ratio method is not suitable for experiments where optimized feeds are not provided. Long-term trials are needed to measure growth performance parameters and RNA/DNA ratios every two weeks to support the results of this study and to improve further research. This simultaneous determination allows a direct comparison of growth parameters and RNA/DNA ratios over a more extended period. It is important to determine whether the two methods stabilize over time and achieve a high level of accuracy even with non-optimized diets.

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CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

TYR and AW designed the study and drafted the manuscript, carried out the study and collected the data; TYR & AW analysed the data; MJS and JH provided laboratory facilities and administrative support; JH and MJS obtained funding for this project and revised and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

Data will be made available on the Pangea data repository at publication.

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