Impact of dietary protein content on growth of white-clawed crayfish *Austropotamobius pallipes* Lereboullet, 1858 (Decapoda: Astacidae) in captive rearing for conservation

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Abstract

The white-clawed crayfish is endangered throughout its entire biogeographic range in Europe due to infection with *Aphanomyces astaci* (crayfish plague) and habitat deterioration. *Ex situ* propagation is of increasing importance to safeguard genetic resources and enable restocking measures after the pathogen's disappearance. This study aimed to quantify the impact of crude protein content on growth response and survival of white-clawed crayfish as optimal growth efficiency of white-clawed crayfish captive rearing has remained largely unexplored. Three separate 80 day feeding trials were conducted: juvenile monosex and juvenile mixed sex crayfish fed medium and high protein diets, and mixed sex adult crayfish fed low and high protein diets. Survival, weight gain and carapace length gain were measured bi-weekly during the trials.

Growth response as the relative gain in cephalothorax length in juvenile mixed sex assemblages was significantly higher for the medium protein diet (*Mdn*=19.4) than for the high protein diet (*Mdn*=15.2, *U*=7.84, *p*=0.009) with an average carapace length increase of 18.3% compared to 16.1% for the medium and high protein diet respectively. However, there was no significant difference in the relative weight gain of juvenile mixed sex assemblages. All data for weight gain and carapace length gain for the juvenile monosex assemblages and adult mixed sex assemblages were not significant in response to diets. Thus, there was no obvious advantage of a high protein diet in the captive rearing of *A. pallipes* and in the case of the juvenile mixed sex assemblage the higher protein content can reduce growth in terms of size.

1. Introduction

The white-clawed crayfish *(Austropotamobius pallipes,* Lereboullet, 1858*)* belongs to the Astacidae*,* an exceptionally diverse group of decapod crustaceans (Crandall and Buhay, 2008; Souty-Grosset and Fetzner, 2016). It is regarded as a keystone species and ecosystem engineer (Momot, 1995; Reynolds and Souty-Grosset, 2011) with an essential role in the trophic dynamics of lentic and lotic freshwater ecosystems (Usio and Townsend, 2002; Reynolds *et al*., 2013).

For decades *A. pallipes* has suffered drastic losses in population numbers coupled with a severe reduction in its geographic distribution (Gouin, 2003; Holdich *et al*., 2009; Kouba *et al*., 2014) resulting in classification as 'endangered' by the *IUCN* in Appendix III of the Bern Convention and Annexes II and V of the EC Habitats Directive (Füreder et al. 2010). This decline is attributed to multiple threats, including fragmentation or destruction of aquatic habitat; flow modification; physical alteration and overexploitation (Grandjean and Souty-Grosset, 2000; Mazza *et al*., 2011). Furthermore, the introduction of non-indigenous crayfish species (NICS), notably the signal crayfish, *Pacifastacus leniusculus* Dana, 1852 often act as vectors for the crayfish plague (*Aphanomyces astaci* Schikora), a virulent oomycete pathogen, which is usually fatal to *A. pallipes* and thus responsible for eradicating entire populations except for one in Catalonia, Spain, with an apparent level of immunity against the pathogen (Reynolds, 1998; Souty-Grosset *et al*., 2006; Martín-Torrijos et al., 2017). Worryingly crayfish plague outbreaks among *A. pallipes* have also been recorded in the apparent absence of NICS. In Ireland there have been at least nine such outbreaks in rivers across the country since 2015 (National Parks and Wildlife Service, Department of Culture, Heritage and the Gaeltacht and Marine Institute, 2019).

Ex situ propagation could conserve and enhance the stock of *A. pallipes,* particularly where its populations are under threat of eradication by plague. However, due to the lack of economic interest in this crayfish species, there have only been very few studies on the captive rearing of *A. pallipes* (Pérez *et al.*, 1998; Sáez-royuela *et al.*, 2002; Carral *et al.*, 2004; Paglianti and Gherardi, 2004; Scalici and Gibertini, 2007; Policar *et al.*, 2009, 2010, 2011). Current knowledge in regard to the nutritional needs of captive reared *A. pallipes* for optimal growth efficiency, in particular, still remains sparse. Wild *A. pallipes* occupies the trophic levels of an opportunistic primary or secondary consumer, feeding on aquatic vegetation, zooplankton, benthic invertebrates and microbially-enriched detritus (Holdich and Lowery, 1988; Momot *et al*., 1978). Any food preferences the animals may exhibit tend to change in accordance with age, physiological state and season of the year where juveniles feed on vegetation, invertebrates, or a combination of both with a shift to a more plant-based diet as they became larger (Gherardi *et al*., 2004; Parkyn *et al*., 2001; Reynolds and O'Keeffe 2005). Therefore, this study aimed to evaluate the effects of dietary protein level effects on growth and survival of *A. pallipes* juveniles in mixed or monosex holding conditions and adult crayfish in mixed sex assemblages.

2. Materials and methods

2.1 Culture conditions

Three 80 day feeding trials with *A. pallipes* were conducted at Cloughram Crayfish, Dundrum, Northern Ireland. This is a purpose-built crayfish aquaculture facility capable of providing test individuals from its own captive rearing program. The facility housed a recirculating aquaculture system (RAS), with water recirculation through a mechanical filter, foam fractionator and moving bed filters for water treatment, at a flow rate of 0.05 L s^{-1}. The water had been abstracted from the Moneycarragh River, Dundrum (Co. Down, Northern Ireland). Daily addition of 150 L river water (1.03% of total RAS water volume) compensated for water loss through feed waste removal and evaporation. Water temperature, dissolved oxygen saturation (DO), and electrical conductivity κ_{25} were monitored with a multi-parameter sonde YSI® 6920 for trials 1 and 2 and Hach® LDO and Hach® sension 156 for trial 3. Values for arithmetic means ± standard deviation were 19.5 ºC (±0.9), 85% DO (±13), 319 µS cm^{-1} (±5), for trials 1 and 2; 19.7 ºC (±0.9), 87% DO (±4), 285 µS cm^{-1} (±6), for trial 3. All trials were carried out in a 12 h-light / 12 h-dark photoperiod setting.

2.2 Experimental diets

Depending on the trial, crayfish were fed one of three commercial dry diets, which differed in CP contents (Table1). Commercial diets were selected as few conservation hatcheries have the resources to make their own food. High protein diet used in all three trials was "Skretting Elite Trout Pellets" (Skretting, Stavanger, Norway) contained 45% CP (high protein, 60% from fish sources) Medium protein diet used in both juvenile trials was "Krmivo SAK Caridina excellent" ™ (Exot Hobby s.r.o., Černá v Pošumaví, Czech Republic) was formulated with 38% CP (medium protein, 50% plant sourced). Low protein diet used in the adult crayfish trials was “King British® Plecostomus tablet food” (King British, Gainsborough, UK) contained 33% CP (low protein, 65% fish sourced and 35% plant based). Low protein diet was not tested with juvenile crayfish due to the relatively high protein requirement of juveniles observed in wild stocks and the risk of mortality if their protein requirements were not met. Low protein diets were used with adult crayfish to investigate the potential implications of the shift in diet requirements with age that has been observed in wild crayfish.

All animals in the test had been reared in the hatchery. Before trials commenced, both juvenile and adult crayfish were randomly selected from their cohort-specific tanks. Test animals were sexed and marked with colour symbols for individual identification. Excess water was removed with tissue paper from each crayfish, before the specimen was weighed to the nearest 0.01 g wet weight with an electronic balance (Precisa 100M-300C). Prior to random transfer into treatment groups, each individual’s cephalothorax length was measured to the nearest 0.01 mm from the tip of the rostrum to the posterior margin at the dorsal midline (Fitzpatrick, 1977) using a digital caliper (Linear Tools MLCP01 calibrated). Individuals (juveniles and adults) were subdivided into fibre glass tanks (43 × 250 × 50 cm) with a bottom surface area of 1.25 m^2 containing approximately 260 L of water. In order to minimise stress, crayfish within each treatment were provided with sheltering tunnels (L: 10 cm, W: 10 cm, H: 5 cm), consisting of 10 cm diameter PVC pipe sections that had been cut in halves, so that each individual animal could make avail of at least one such shelter. Moulting and survival of each individual was monitored during the trials.

During trials individuals were caught and identified once every 14 days, when weight and cephalothorax lengths were recorded as biometric data to determine growth response. Juvenile crayfish were fed twice a day to apparent excess with daily feed rations initially set at 8% total crayfish biomass in each experimental treatment and subsequent adjustments according to consumption and increased growth rate after 14 days. Adult crayfish were fed in excess once a day, with an allocation of 15 g feed per treatment group and day. Waste and uneaten food were removed from each treatment every two days using a siphon vacuum.

2.3 Juvenile crayfish experiments

A total of 240 juvenile (1+ class) *A. pallipes* (initial mean weight 1.73 g ± 0.39; initial cephalothorax length 17.40 ± 1.39 mm) were fed a medium (38%) or high protein (45%) diet, in order to quantify the impact of protein content on survival and growth response. As crayfish can be aggressive towards each other, juvenile trials also provided an opportunity for the investigation of effects of diet from holding them in monosex versus mixed sex assemblages.

2.3.1 Trial 1: Mixed sex groups

A total of 120 juvenile crayfish was divided into two feeding groups and two separate tanks (arithmetic means ± standard error: Initial weight 1.59 ± 0.15 g for females, 1.62 ± 0.09 g for males; initial cephalothorax length 17.40 ± 0.31 mm for females, 17.28 ± 0.18 mm for males). Stocking density was 48 m^{-2}. For this trial 30 male crayfish and 30 female crayfish received a high protein diet (45% CP) and 30 male crayfish and 30 female crayfish received the medium protein diet (38% CP).

2.3.2 Trial 2: Monosex groups

A total of 120 juveniles (arithmetic means ± standard error: Initial weight 1.71 ± 0.04 g for females, 1.95 ± 0.05 g for males; initial cephalothorax length 17.39 ± 0.15 mm for females, 17.88 ± 0.20 mm for males)was held in monosex groups, with one male and one female group for each diet. These four groups were housed in separate tanks, with a stocking density of 24 m^{-2}. For this trial 30 male crayfish and 30 female crayfish received the medium protein content (38% CP); 30 male crayfish and 30 female crayfish received a high protein diet (45% CP).

2.4 Adult crayfish experiments

Trial 3: Adult crayfish

In total 120 adult *A. pallipes* were divided into two feeding groups with mixed sexes (arithmetic means ± standard error: Initial weight 10.10 ± 0.62 g for males and 7.52 ± 0.43 g for females; initial mean cephalothorax length 31.70 ± 0.63 mm for males and 29.14 ± 0.52 mm for females). Feeding groups were kept in separate tanks with a stocking density of 48 m^{-2}. For this trial 30 male crayfish and 30 female crayfish received low protein feed (33% CP) and 30 male and 30 female crayfish received high protein feed (45% CP). Single sex trials were not conducted with adults as the animals were kept in mixed sex groups to prepare for winter breeding.

2.5 Statistical analysis

Upon completion of each trial, growth performance for each individual was evaluated as a function of the overall increase in weight and cephalothorax length and a measure of the following parameters in accordance with studies by (Cortés-Jacinto *et al*., (2003) and Fuertes *et al*., (2013):

(**i**) Relative weight gain (RWG) = $(W_t - W_i) / W_i \times 100$

(**ii**) Relative cephalothorax length increase (RCI) = $(C_t - C_i) / C_i$ x 100

(**iv**) Survival rate (SR) = $(N_t - N_i)$ x 100

W_t represents the final weight and W_i is the initial weight; C_t represents the final cephalothorax length and C_i is the initial cephalothorax length; D_t is the total amount of feed administered to a treatment; N_t represents the number of a treatment's surviving crayfish at the end of a trial and N_i is the number of individuals at the trial start; t is the duration of the trial in days (80 days for all trials in this study).

All data are presented as arithmetic means ± standard error. For the relative weight gain and relative carapace length increase, differences between all treatments in individual trials were tested for normality with a Kolmogorov-Smirnov test and assessments of z-scores for skewness and kurtosis (Kim, 2013). Statistical analysis of these two measures by Mann-Whitney U tests was completed for data with strong departures from normality or two-way ANOVA and multi range analysis with a Tukey test for data with normal distributions. The selected level of significance was α=0.05. Two-way ANOVA was performed for to investigate single and combined effects of sex and diet. All statistical analyses were carried out in IBM SPSS Statistics ®.

3. Results

3.1 Trial 1: Mixed sex groups (Juveniles)

Survival rates, relative gains in weight and cephalothorax length increase for mixed sex assemblages (n=60) are presented in Table 2. In comparison to the high protein diet, both sexes achieved larger increases in cephalothorax length with the medium protein feed with an average combined increase of 16.1% and 18.3% for the high and medium protein diet respectively; weight gain of females was also higher with this diet with an average combined increase of 69.0% and 71.4% for the high and medium protein diet respectively. Data for relative cephalothorax length increase in percent deviated from a normal distribution. A Mann-Whitney U test indicated that for this growth parameter the increase was significantly higher for the medium protein diet (Mdn=19.4) than for the high protein diet (Mdn=15.2) U=7.84, p=0.009. Weight gain data were normally distributed. For the difference in relative weight gain between juveniles in mixed sex groups the level of significance was missed by a small margin (F=3.71; df=1,96; p=0.057). The interaction between sex and diet was not significant for relative weight gain (F=0.35; df=1,96; p=0.558); both sexes achieved higher increases with the medium protein diet. During this trial 90% of crayfish moulted twice, 6% moulted three times and the remaining animals moulted once. Survival rates were the same in treatments with both dietary regimes (83%).

3.2 Trial 2: Monosex groups (Juveniles)

Survival rate, relative weight gain and relative cephalothorax length increase for monosex assemblages of juveniles (n=30) are presented in Table 2. The relative weight gains increase in treatments containing monosex assemblages showed male crayfish increased in weight by an average of 70% and 75% while female crayfish increased by 70% and 73% for the high and medium protein diets respectively. These differences in relative weight gain were significant between sexes (F=22.11; df=1,93; p<0.001) but not for diet (F=0.51; df=1,93; p=0.477). The relative cephalothorax length increased by an average of 19% and 20% for male crayfish and by 17% and 19% for female crayfish fed the high and medium protein diets respectively. There was no significant difference between relative cephalothorax length increase for sex (F=4.63; df=1,93; p=0.054) or diet (F=1.45; df=1,93; p=0.231) with juvenile crayfish held in monosex assemblages. The interaction between sex and diet was only significant for weight gain (F=13.78; df=1,93; p<0.001); During the trial 92% of crayfish moulted at least twice, 5% moulted three times and the remaining animals only moulted once. Survival rates were found to be lower in male monosex assemblages compared to female monosex assemblages. Pairwise comparisons within the same gender for increases in relative cephalothorax lengths or relative weight gain indicated no significant differences between growth in mixed sex and monosex trials.

3.3 Trial 3: Adult crayfish

Survival of the test organisms exceeded 76% in all treatments but was higher for male crayfish fed the low CP diet (Table 3). The mean male cephalothorax was around 3 mm longer than the mean for females and increased by 9% on both diets compared to 6% and 7% for the female crayfish fed high and low protein diets. The mean male was also 4.0 g heavier by comparison and increased in weight by more than 30% while females gained less than 20%, regardless of the diet. In relative size, differences between sexes remained significant throughout the trial for relative cephalothorax length increase (F=6.59; df=1,99; p=0.012) and relative weight gain (F=14.08; df=1,99; p<0.001). However, differences between the two diets were not significant in this respect (weight gain F=0.02; df=1,99; p=0.903; cephalothorax length increase F<0.01; df=1,99; p=0.988). The interaction between sex and diet was neither significant for cephalothorax length increase nor for weight increase (carapace length $F=0.22$; $df=1,99$; $p=0.641$; weight gain $F=0.31$; $df=1,99$; $p=0.579$); growth of both sexes was independent of the diet.

While weight increase of crayfish in this trial was gradual, the cephalothorax length did not increase simultaneously; growth spurts in regard to this parameter were observed after moulting events. There was a frequent occurrence of moults during the trials. Moulting was observed for 88% of the animals, and 7% of the crayfish specimens moulted twice during the trial. There was also evidence of cannibalism, particularly in the treatment with the high protein diet, where remains of dead crayfish were repeatedly seen.

4. Discussion

This study investigated the impact of protein content on growth response as an important aspect in the development of practical diets for *A. pallipes*. In conventional aquaculture, cost considerations and dietary requirements are important criteria for the selection of crayfish feed. Due to the apparent paucity of studies on the aquaculture of *A. pallipes*, it is difficult to find published growth rates for comparison. The majority of previous crayfish diet research has focused on species with greater commercial potential such as *P. leniusculus* (signal crayfish; Harlıoğlu, 2009) or *Cherax quadricarinatus* (redclaw crayfish), whose high fecundity, elevated growth rate and large size make their use in aquaculture more appealing (reviewed by Holdich, 1993). Reported growth rates of these commercially important species are typically 10-18 times higher for *C. quadricarinatus* and *P. leniusculus* respectively (Cortes-Jacinto *et al*., 2004; Carral *et al*., 2011) than those recorded for *A. pallipes* in this study.

The similarity of growth and survival rates of juvenile *A. pallipes* on medium and high protein diets suggests that an increase of dietary CP content beyond 38% would not result in improved outcomes of their captive rearing.

This corresponds well to other nutritional studies on European Astacidae. Ghiasvand *et al*. (2012) found that juvenile, narrow clawed crayfish (*Astacus leptodactylus*) on a diet of 30% CP achieved similar growth rates to those on a diet of 40% CP. Abeel *et al.* (2014) reported that the optimum protein concentration required for maximum growth and survival for juvenile *A. astacus* (+1 year class: 3.18 ± 1.05 g) housed in a closed recirculating system consisted of 35% CP (principally formulated from fish meal) and Ackefors *et al*. (1992) suggested that the optimum protein level for captive rearing of *Astacus astacus* juveniles in a closed recirculating system ranged between 30-40% animal protein, while crayfish older than one year could be adequately sustained on protein levels as low as 20%.

Wild *A. pallipes* specimens usually reach sexual maturity within three to four years, at approximately 50 mm total length, or at >25 mm carapace length, but this can be reached at 16 months in captive breeding (Holdich, 2003; Nightingale *et al.* 2018).

As expected, juvenile *A. pallipes* in this study achieved higher growth rates than adults, and adult males grew larger than females. These characteristics also apply to populations in natural environments. In the River Ouse (Buckinghamshire, England) *A. pallipes* juveniles of both sexes exhibited similar patterns of moult frequency and growth before reaching sexual maturity (Pratten, 1980). During adult life, growth rates of male *A. pallipes* individuals from a population in Northumbria also exceeded those of females (Brewis and Bowler, 1982). The methods undertaken within this investigation saw juvenile crayfish fed a diet consisting of 38% and 45% crude protein while the adult crayfish were fed a diet with 38% and

33% crude protein. The lower crude protein diets from each trial were found to provide sufficient growth. Adult crayfish received lower protein content than juveniles as their life stage mean they have different demands for protein. This was previously observed by Reynolds and O'Keeffe (2005) who found juveniles *A. pallipes* fed on vegetation, invertebrates, or a combination of both with a shift to a more plant-based diet as they became larger. Due to the high monetary cost of protein-rich diets, this investigation aimed to use a lower crude protein diet without harming or restricting growth. As a result, adults were feed a more cost effective 33% crude protein diet which reflected their natural diet while also reducing expenditure on conservation effort. During the trials, the inhabitants of one tank were fed each of the crude protein diets. Ideally, this would need to be repeated with multiple tanks consisting of crayfish fed on the same diet. However, this was not possible owing to several constraints which included hatchery size and the number of crayfish housed within it along with the sourcing of additional stock which was not permitted.

In captive rearing of crayfish, stocking density can have a significant impact on production parameters, such as survival and growth, particularly within closed circulatory systems (Lutz and Wolters, 1986; Naranjo-Páramo *et al.*, 2004). Nightingale *et al.* (2018) reported no detrimental effect on survival for high stocking densities of up to 300 juveniles m^{-2} but observed the best growth at a 100 m^{-2} density. Stocking densities in this study were 48 m^{-2} for adult trials and juvenile mixed sex trials, and 24 m^{-2} for juveniles monosex trials. Low stocking densities were used to prevent conflict for food and thus provided optimal conditions for growth aiming to maximise the survival of this endangered species under hatchery rearing. Different stocking densities were used in the monosex trail as it was not possible to reduce the tank size in the pre-build hatchery to allow for the same stocking density. The survival of *A. pallipes* ranged between 77% - 84%. This was relatively high even in comparison to reported values for the Australian red claw crayfish *Cherax quadricarinatus* as a very successful invasive species, e,g. Jones and Ruscoe (2001) 43%, Pinto and Rouse (1996) 72% and Jones and Ruscoe (2000) 76% - 87%, but lower than the mean survival 87.7% ± 2.8% reported by Nightingale et al. (2018) for *A. pallipes* juveniles. Crayfish are aggressive in nature and studies indicate that provision of shelter protects against predation and minimises aggressive interactions. This is particularly important in periods of increased vulnerability during moulting and mitigates a potential negative impact of high stocking densities (Jones and Ruscoe, 2001). This study provided one shelter for each individual; this may account for the higher survival rates compared to other studies on captive rearing, e.g. Ulikowski and Krzywosz, (2006) recorded a survival rate of 16 - 24% in juvenile narrow-clawed crayfish (*Astacus leptodactylus*) when no or only a small number of shelters were provided.

Other measures to consider for further improvement of survival rates in the rearing of *A. pallipes* would be the adjustment of stocking densities as the animals grow and demand more space; optimum stocking densities typically decrease with crayfish size (Abeel *et al*., 2012). Size differences of crayfish in holding tanks increase the risk of smaller individuals succumbing to aggression and cannibalism of larger specimens and have a negative impact on growth (Houghton *et al.,* 2017; Romano and Zeng, 2017). In feeding trials with adult *A. pallipes* cannibalism did occur, but it was unclear if the cause of death was related to aggression or whether these were observations of opportunistic feeding on a carcass. For other species like *P. leniusculus* cannibalistic behaviour appears to be linked to an individual's size, as it has been more commonly observed for larger crayfish (Houghton *et al.,* 2017); hence we separated our captive crayfish based on age and size. Furthermore, aggressive behaviour between different size classes does not only affect survival, but it can also affect growth rates. González *et al*. (2011) recorded significantly lower feed conversion rates for size graded groups of *P. leniusculus* (mean 2.64 g) than for ungraded groups (mean 3.23 g) and concluded that crayfish grading enables better performance and improves overall feeding efficiency in aquaculture. Nevertheless, the absence of significant differences of growth responses between monosex and mixed sex diets in the juvenile *A. pallipes* trials suggests that at this early stage social dominance did not play a substantial role for animals of this crayfish species, perhaps due to an ample supply of feed.

A. pallipes in these trials were fed with protein that was partly sourced from fish meal. Future research on the captive rearing of this species should investigate the feasibility of feeding regimes solely based on plant-based proteins. The motives for replacing fish meal in aquaculture are improved sustainability through independence from the exploitation of ocean fish and potential cost (Fuertes *et al*., 2012; Naylor *et a*l., 2009).

Control over individual ingredients through feeding specifically formulated diets would overcome the limitations imposed by this study's reliance on commercial feeds.

5. Conclusion

Currently there is no commercial interest in captive rearing of *A. pallipes* for food production, but e*x situ* propagation can help to safeguard this species against the threat of its extermination due to crayfish plague. The comparison of commercial diets with different CP levels for feed optimisation of juvenile and adult captive reared white-clawed crayfish did not provide any evidence for a benefit of diets with higher protein levels. For juvenile *A. pallipes*, a medium protein diet of 38% CP yielded an equally strong growth response as a high protein diet of 45% CP. Furthermore, the medium CP diet significantly increased the relative cephalothorax length gain in mixed sex assemblages of juvenile crayfish. For adults, a low protein diet of 33% CP proved as effective as the high protein diet of 45% CP for the growth of the different sexes.

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Table 1. Composition of the experimental diets for *A. pallipes* in % dry mass. High and medium protein diets were used for trials 1 and 2 with juvenile crayfish (monosex and mixed sex); low and medium protein diets were used for trial 3 with adult crayfish (mixed sex).

	High-Protein (45%)	**Medium-Protein (38%)**	**Low-Protein (33%)**
	(%)	(%)	(%)
Crude oils & fats	20	18	4
Crude Protein	45	38	33
Crude Ash	12	11.8	32
Carbohydrates	19.1	22.5	25.2
Crude Fibre	2	8	1
Calcium	0.8	0.6	0.8
Phosphorus	0.8	0.8	0.1
Sodium	0.3	0.3	0.2
Trial	1, 2, 3	1, 2	3

Table 2. Survival and growth response values of juvenile *A. pallipes* in trial 1 and 2 on medium and high CP diets over 80 days (mean ± standard error), where RWG = relative weight gain, RCI = relative cephalothorax length increase, DW = average daily weight gain. Superscript letters indicate significant differences in the RWG or RCl

	High Protein Content			**Medium Protein Content**		
Trial 1 – mixed sex	Male	Female	Combined	Male	Female	Combined
Survival (%)	83	83	83	83	83	83
RWG (%)	69.6 ± 1.9[a]	68.4 ± 1.9[a]	69.0 ± 1.3[a]	71.9 ± 1.9[a]	70.9 ± 2.6[a]	71.4 ± 1.6[a]
RCI (%)	16.3 ± 0.9[a]	15.9 ± 1.0[a]	16.1 ± 0.6[a]	18.4 ± 1.2[b]	18.3 ± 0.9[b]	18.3 ± 0.8[b]
DW (mg)	13.8 ± 0.5	13.0 ± 0.6	13.4 ± 0.4	13.8 ± 0.8	14.5 ± 0.5	14.1 ± 0.5
Trial 2 – monosex	Male	Female		Male	Female	
Survival (%)	78	84		81	84	
RWG (%)	69.8 ± 0.0[a]	70.0 ± 1.7[b]		74.7 ± 2.0[a]	72.5 ± 0.0[b]	
RCI (%)	18.7 ± 0.9[a]	17.3 ± 0.9[a]		20.2 ± 0.9[a]	19.1 ± 0.0[a]	
DW (mg)	16.4 ± 0.6	15.9 ± 0.5		18.6 ± 0.4	14.5 ± 0.5	

Table 3 Survival and growth response of adult *A. pallipes* in trial 3 with mixed sex groups on low and high CP diets over 80 days (mean ± standard error), where RWG = relative weight gain, RCI = relative cephalothorax length increase, DW = average daily weight gain. Superscript letters indicate significant differences in the RWG or RCl

	High Protein Content		**Low Protein Content**	
	Male	Female	Male	Female
Survival (%)	87	77	97	83
RWG (%)	32.6 ± 3.4^{a}	19.6 ± 2.8^{b}	35.3 ± 5.4^{a}	17.8 ± 2.2^{b}
RCI (%)	9.1 ± 0.8^{a}	6.4 ± 1.0^{b}	8.9 ± 1.4^{a}	7.0 ± 0.8^{b}
DW (mg)	34.1 ± 4.6	13.2 ± 2.6	39.8 ± 5.7	13.2 ± 2.0