Cent. Eur. J. Biol. • 9(1) • 2014 • 1-10
DOI: 10.2478/s11535-013-0134-0

VERSITA

Central European Journal of **Biology**

Protein synthesis in crustaceans: a review focused on feeding and nutrition

Review Article

Chris G. Carter[1,*], Elena Mente[2]

[1]*Institute for Marine and Antarctic Studies, University of Tasmania, TAS 7001 Hobart, Australia*

[2]*School of Agricultural Sciences, Department of Ichthyology and Aquatic Environment, University of Thessaly, GR-38446 N Thessaly, Greece*

Received 24 August 2012; Accepted 04 December 2012

Abstract: This review aimed to place crustacean research on *in vivo* protein synthesis into a broader context, assess its potential for providing further insights into crustacean nutrition and physiology, and recommend future directions relevant to crustacean aquaculture. In crustaceans the flooding dose measurement of protein synthesis is the only method that has been used, it is relatively complex, time consuming and uses radioactive labels. Protein synthesis provides a subtle approach to assessing imbalances and deficiencies in dietary amino acid and energy. In addition, the calculation of protein synthesis retention efficiency (SRE) is recommended in order to understand and optimize parameters such as feeding regime and diet composition. For prawns, SRE was highest at optimum dietary protein content and quality. Similarly the most efficient feeding regimes in juvenile lobsters were demonstrated by the highest efficiency of retaining synthesized protein. Understanding how various abiotic and biotic factors influence protein synthesis has great potential for improving different aspects of crustacean aquaculture but very few studies have done this; better knowledge of how abiotic and biotic factors affect crustacean protein synthesis will contribute to optimising growth of crustaceans in culture.

Keywords: *Fisheries • Aquaculture • Crustaceans • Protein metabolism • Protein synthesis*

1. Introduction

Protein synthesis is central to growth and renewal in all organisms and has been investigated across taxa, in relation to numerous variables and using a variety of methods [1]. Understanding protein synthesis at the tissue and organism level has revealed much information about animal physiology and provided new insights into many biological questions ranging from fundamentals of growth to applied issues that relate feeds and nutrition [1-3]. Research on ectothermic aquatic animals has mainly concerned fishes, less has been done on invertebrates and the literature on crustaceans is comparatively small and reflects nodes of activity by a few researchers. In this review protein synthesis refers specifically to the *in vivo* measurement of amino acid incorporation from a labeled pre-cursor pool at the level of the whole animal and tissue following the general approach adopted previously for understanding animal physiology and nutrition [2,4,5]. The general approach considers the flux of protein into and through the whole animal and expands on the energy and nitrogen budget concept to incorporate protein synthesis in tissues and at a whole animal level of organization [6-8]. Key components are protein intake, protein digestibility, amino acid absorption, tissue free amino acid pools, protein synthesis, protein degradation, ammonia excretion, and protein retention as protein growth [8-10]. Relationships between intake, synthesis and retention describe levels of efficiency of protein use and provide important comparative indices including synthesis retention efficiency and will be discussed further [2,4,5].

Several reviews have been written about protein turnover in ectotherms [2,5,11-14], only one was written

* *E-mail: chris.carter@utas.edu.au*

in the last 10 years [4] and only one was specifically on crustaceans with a focus on temperature, specific dynamic action (SDA) and protein synthesis [15]. To the best of our knowledge, fewer than 20 studies have been published on protein synthesis in crustacean species over the last 25 years (Table 1). The literature is summarized in relation to the species, the life-cycle stage and weight, period in the moult cycle and the method used for measuring protein synthesis (Table 1). Isopods, prawns, crabs and lobsters have been investigated using mainly small juveniles, with sizes ranging from 0.2 g post-larval European lobsters (*Homarus gammarus*) to adult 100 g adult shore crabs (*Carcinus maenus*) [16,17]. Early studies measured incorporation of radioactive amino acids using "pulse labeling" [18,19] after which the majority used a "flooding dose" of a radioactive amino acid (^{3}H-phenylalanine) to calculate fractional rates of protein synthesis [16]. Main areas of interest have been in the moult cycle, and the effects of environmental and nutritional variables (Table 2).

Species[1]	Stage and weight	Moult period	Method and variables studied	Reference
Carcinus maenas	Adult	PR, PO, I	FD, inject third walking leg	[16]
Green crab	50-100 g		Moult cycle: Muscle fibre type	
Carcinus maenas	Adult	I	FD, inject third walking leg	[25]
	30-70 g		Feeding: SDA, fast, re-feed	
Carcinus maenas	Adult	I	FD, inject third walking leg	[41]
	39-67 g		Environment: Hypoxia	
Homarus americanus	Juvenile	PR, PO, I	FD, inject walking leg, also *in vitro* for muscle	[24]
American lobster			Moult cycle: Muscle type. Hormone (20-HE)	
Homarus gammarus	Post-larvae	I	FD, inject abdomen	[17]
European lobster	0.16 g		Feeding: ration and feeding regime	
Nephrops norvegicus	Adult (male)	I	FD, inject abdomen	[26]
Norway lobster	30-60 g		Feeding: dietary regime, starvation	
Penaeus vannamei	Post-larvae	Complete	^{3}H-leucine incorporation, protein synthesis inhibitor	[20]
Whiteleg shrimp			Moult cycle: whole animal	
Penaeus vannamei	Juvenile	I	FD, inject abdomen	[31]
	1-2 g		Dietary protein and essential amino acids	
Penaeus esculentus	Juvenile	I	FD, inject abdomen	[27]
Brown tiger prawn	5 g		Dietary protein (30, 40, 50%)	
Macrobrachium rosenbergii	Post-larvae, Juvenile	I	FD, inject pericardial region	[44]
Giant river prawn	0.4g, 1.3g		Salinity (14.30 ppt) Temperature (20, 26, 30°C)	
Saduria entomon	Adult (male)	I	FD, inject pericardial region	[36]
Temperate isopod	2.8-5.2 g		SDA. Temperature (4, 13°C)	
Glyptonotus antarticus	Adult (male)	I	FD, inject pericardial region	[30]
Antarctic isopod	32.5 g		SDA. Temperature (0, 4°C)	
Glyptonotus antarticus *Idotea rescata*	Adult	I	FD, inject haemocoel	[34]
			Temperature (0, 4, 14°C)	
Ligia oceanica	Adult (male)	I	FD, inject haemocoel	[40]
Temperate isopod	>0.20 g		Temperature (5-25°C)	

Table 1. Protein synthesis in crustacean species.

[1] *FAO (2012). I, intermoult; PR, premoult; PO, postmoult. FD, flooding dose of 3H-phenylalanine.*

Species	Variable	Tissue (time of measurement)[1]		Reference
		k_s (% d^{-1})		
Carcinus maenas	Tissue, feeding regime and SDA	Claw muscle	HP	[25]
	Unfed (24 h)	0.15	2.5	
	2.6 % BW meal (3 h)	0.55	9.5	
	2.6 % BW meal (9 h)	0.37	3.5	
	2.6 % BW meal (16 h)	0.33	3.7	
		Whole animal	Whole animal (integrated)	
	Unfed (24 h)	1.6	2.56	
	2.6 % BW meal (3 h)	3.0		
	2.6 % BW meal (9 h)	2.9		
	2.6 % BW meal (16 h)	2.7		
Homarus gammarus	Ration and feeding regime	Whole animal (24 h)		[17]
	20% BW d^{-1} (daily)	8		
	10% BW d^{-1} (daily)	8		
	10% BW d^{-1} (20% every 2 days)	8		
	5% BW d^{-1} (daily)	13		
	5% BW d^{-1} (20% every 4 days)	12		
Nephrops norvegicus	Feeding regime	Tail muscle (24 h)	HP (24 h)	[26]
	Fed in laboratory	0.35	7.55	
	Starved in laboratory	0.21	1.01	
	Wild-caught	0.35	2.88	
Penaeus vannamei	Dietary protein source	Whole animal (24 h)	Whole animal (integrated)	[31]
	Marine proteins	9.8	24.3	
	Marine proteins plus soy	9.6	23.9	
	Purified (casein)	9.1	22.5	
Penaeus esculentus	Dietary protein content	Muscle (24 h)	HP (24 h)	[27]
	30% protein	1.4	58	
	40% protein	1.0	53	
	50% protein	1.5	50	
Saduria entomon	SDA and temperature	Whole animal (24 h)	Whole animal (SDA peak)	[36]
	Normal temperature (4°C)	0.57	1.53	
	Elevated temperature (13°C)	1.36	2.64	
Glyptonotus antarticus	SDA and temperature	Whole animal (24 h)	Whole animal (SDA peak)	[30]
	Normal temperature (0°C)	0.16	0.38	
	Elevated temperature (4°C)	0.22		

Table 2. Rates of protein synthesis in crustaceans according to whole animal and tissue type and in relation to experimental variables studied (HP, hepatopancreas).

[1]Rates measured 24 h after feeding (24 h), at the predicted peak rate after feeding (SDA peak), or integrated over 24 h after feeding (integrated).

The aim of this review is to place crustacean research on protein synthesis into a broader context, assess the potential of measuring protein synthesis for providing further insights into crustacean nutrition and physiology, and recommend future directions that are relevant to crustacean aquaculture. Reference to relevant research on fish and other animal taxa will be made in order to understand differences and similarities with crustaceans.

2. Methods and approaches

Early studies measured incorporation of radioactive amino acids using "pulse labeling", whilst informative this measures the net accumulation of the labeled amino acid in protein rather than the overall amount of protein synthesized [18-22]. Three methods are used to measure *in vivo* protein synthesis and termed constant infusion, flooding-dose and stochastic endpoint [4,5]. The majority of research on aquatic ectotherms and all the studies on crustaceans used a flooding-dose of radioactive amino acid (^{3}H-phenylalanine) according to the method introduced by Garlick *et al.* [23] for work on rats. The correct application and validation of the flooding-dose method with aquatic ectotherms is fully discussed in previous reviews [4,5]. The first use of a flooding-dose with a crustacean was to make a very complete analysis of muscle protein synthesis in relation to the moult cycle of the shore crab [16]. This and other early studies on crustaceans used a flooding-dose to investigate *in vitro* as well as *in vivo* tissue protein synthesis, *in vitro* rates were lower than *in vivo* rates [16,24] and will not be discussed further.

A flooding-dose is applied by injection and *in vivo* protein synthesis measured over a period of around an hour. One hour incorporation is similar for many ectotherms, including fish and molluscs; temperature has a critical effect so that incorporation is faster at higher temperature and slower at lower temperatures [4]. A suitable incorporation time range should be validated for each species and particularly in relation to the experimental temperatures which will clearly have a major influence on rates of physiological processes including protein synthesis. Injection into the haemolymph has been *via* the sinus of various limbs [16,25] or into abdominal segments [26,27]. Measurements of the specific activity of the radioactive label in the free amino acid pool and bound into the tissue protein provide a fractional rate of protein synthesis that is scaled up to 1 day (k_s; % d^{-1}). This expresses the amount of protein synthesized in relation to the total of amount of protein in the relevant tissue or in the whole animal. Information on fractional rates of protein synthesis in different tissues and in relation to experimental variables is summarized in Table 2.

Where growth of the animal or tissue is known, fractional rates of protein growth (k_g; % d^{-1}) can be calculated directly from changes in the protein content of whole animal or tissue over a given time. Fractional rates of protein degradation (k_d; % d^{-1}) are calculated indirectly as $k_d=k_s-k_g$ [2,5]. Protein turnover (k_t; % d^{-1}) may also be considered; in growing animals it is equivalent to protein degradation and in animals losing protein it is equivalent to protein synthesis. This is because protein turnover expresses the amount of protein that is "turned over" and replaced from the existing protein pool in the animal [4]. Fractional rates of protein intake (k_c; % d^{-1}) *via* food provide an important dimension to understanding protein synthesis [28].

Two relationships between fractional rates of protein intake, synthesis and growth express efficiencies of protein utilization at various stages in its assimilation: synthesis retention efficiency SRE(%)=100 k_g/k_s; anabolic stimulation efficiency ASE(%)=100 k_s/k_c. Presumably k_c and ASE are analysed more frequently in fish research because feed intake by fish can be measured more accurately than for crustaceans [28]. All studies on crustaceans have considered two further measures of protein synthesis, the ratio between RNA and protein termed the capacity for protein synthesis (C_s, µg RNA mg protein^{-1}) and RNA activity (k_{RNA}, mg protein synthesized mg RNA^{-1} d^{-1}) [5,29]. These measurements can be made at the level of the whole animal and tissues, comparison between tissues has often revealed and explained important differences in rates of protein synthesis. Muscle, usually leg and claw, and the hepatopancreas (digestive gland) are the most studied tissues. As will be discussed below, the simultaneous or concurrent measurement of other parameters, including oxygen consumption [25], ammonia excretion [30], amino acid free pools [31] and hormones [24], have added considerably to the interpretation of protein synthesis data.

3. Moult cycle

Crustacean growth is characterised by moulting, ecdysis, when the old exoskeleton is shed to allow an immediate increase in size and a gradual accumulation of nutrients and energy proceeding the next moult [32]. Initial research on protein synthesis over the moult cycle measured *in vitro* incorporation of radio-labelled amino acid (leucine) by crayfish (*Orconectes virilis*) hepatopancreas [18,19]. This early work was quite

insightful and suggested increases in protein, RNA and RNA synthetic activity during the premoult period. Interestingly, there was no increase in *in vitro* protein synthesis at premoult, whether this was due to the method or the tissue measured remains unclear (see below). The importance of protein synthesis in the synthesis of chitin and crustacean cuticle was established by Horst [20,21] using post-larval prawn species and post-moult crabs. Protein synthesis was, however, not measured directly, *in vivo* rates of incorporation of radio-labelled amino acids were 85-90% lower when the protein synthesis inhibitor puromycin was administered [20].

The direct measurement of protein synthesis over the moult cycle of the shore crab (Figure 1) clearly established that the highest rates occurred during the premoult stages and rates were approximately 15 to 20 times lower during the intermoult period [16]. In the carpopodite extensor muscle, protein synthesis ranged between about 0.1 and 1.7% d^{-1} and was highest immediately prior to moult, intermediate following moult and lowest during intermoult [16]. Interestingly, protein synthesis varied with both muscle fibre type and fibre location within the muscle so that slow-type tonic fibres had rates 2.1 times higher than fast-type phasic fibres and, post-moult, the highest activity was at the external cuticular end of the muscle [16]. The American lobster had a similar pattern amongst 3 muscle types, rates were higher in claw muscle than in leg and abdomen and all muscles had peak rates at premoult whereas rates were not different between immediate postmoult and intermoult [23]. Changes in protein synthesis were mediated through changes in RNA activity rather than RNA content which stayed constant over the moult cycle [23].

These results reinforce the validity of using intermoult animals when investigating the influence of other variables on protein synthesis. They highlight the importance of further studies about protein synthesis during moulting and metamorphosis in crustaceans. Examination of inter-specific differences in life-history patterns of protein synthesis would make a fascinating comparison amongst crustaceans and with cephalopods, a taxa with very different a life-history that grow very rapidly, reproduce in one season and die [33].

4. Feeding

Feeding regime and recent nutritional history, specifically the frequency of feeding and ration consumed, have a strong influence on protein synthesis in all animals including aquatic ectotherms [28]. Two aspects of feeding and their relationship to protein synthesis have received most attention in the crustacean literature, these are specific dynamic action, SDA [15,25,30,34-36], and starvation and re-feeding [17,25,26]. Following a meal the flux of amino acids and other nutrients from the digestive system into the other tissues stimulates a rapid increase in metabolism reflected by increases in oxygen consumption, protein synthesis and ammonia excretion [28,37]. SDA is typically characterised by its "duration" which describes the time taken to return to a pre-feeding value and, as a key component of SDA, protein synthesis responds in the same way to the last meal over time. Duration is strongly influenced by temperature so that it extends for less than a day in most tissues in temperate crustaceans to several days in Polar crustaceans [15]. Several studies have demonstrated that protein synthesis rates generally decrease to a relatively constant level in starved crustaceans. It also appears that the frequency with which crustaceans are fed can affect both protein synthesis and degradation. Rates of both protein synthesis and degradation were elevated in lobsters either fed continually on low rations or fed less frequently [17]. Consequently, protein turnover was higher and growth reduced compared to animals fed more frequently which consumed more food and used it more efficiently [17].

One of the earliest studies on protein synthesis in crustaceans provided a very thorough analysis of SDA in the shore crab [25]. Individual adult crabs were fed similar rations of the same diet and used to measure protein synthesis at 3 different times after feeding in 6 tissues (Figure 2) and in the whole animal. Protein synthesis increased to a strong peak at 3 h in the gill, hepatopancreas, claw and heart muscles whereas the peak was more extended in the leg muscle and

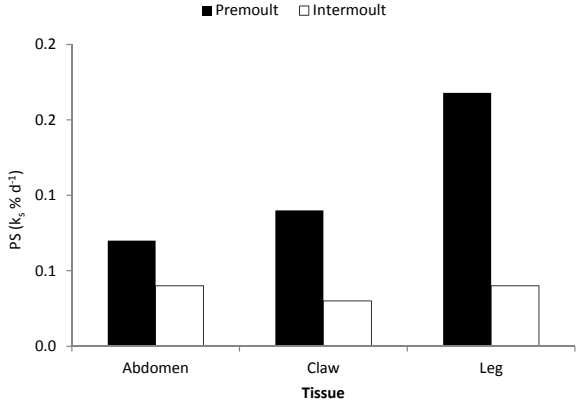

Figure 1. Protein synthesis (PS) expressed as a fractional rate (k_s, % d^{-1}) in muscle tissue from the abdomen, claw and leg of adult green crab in relation to moult cycle, data from [16].

proventriculus. After 16 h rates of protein synthesis had returned to pre-feeding levels in all tissues except for the claw muscle and proventriculus. At their peak, rates were 2-4 times higher, depending on the tissue, than at pre-feeding (Figure 3). There are strong similarities with post-prandial protein synthesis in fish including the rapid peak, its relative magnitude and how this is of longer duration in muscle [38,39]. In continuously fed animals protein synthesis was similar to pre-feeding rates in all tissues except the claw muscle and was similar to the peak rate.

Whole animal protein synthesis in the shore crabs discussed above [25] was related to oxygen consumption in order to determine the contribution to total metabolism and estimate an energetic cost of protein growth. Whole animal protein synthesis reached a peak at 3 h and remained elevated for at least 9 h, oxygen consumption also peaked at 3 h and gradually declined to be approximately 2 times the unfed level after 16 h. This was the first study in crustaceans to show a relationship between protein synthesis and oxygen consumption and also to model the energy cost of protein synthesis, protein synthesis accounted for 20 to 37% of the total oxygen consumption [25], and to show crustaceans were similar to other ectotherms [12]. An interesting set of experiments on Polar and cold water/temperate isopods explored SDA through parallel measurements of oxygen consumption, nitrogenous excretion and protein synthesis [15,30,34,36,40]. The key finding was that, despite differences in the SDA response, the relationship between whole animal oxygen consumption and protein synthesis was similar for an Arctic and an Antarctic isopod [15]. The metabolic cost of protein synthesis was calculated as 141 mmol oxygen per gram of protein synthesised and, as expected, within the range for other animals [15].

Mente *et al*. [41] studied the postprandial changes in O_2 consumption, arterial blood PO2, and tissue protein synthesis in the crab *Carcinus maenas* in normoxic, O_2-depleted, and O_2-enriched waters after feeding. In normoxic water (21 kPa), the arterial PO_2 was 1.1 kPa before feeding and 1.2 kPa 24 h later whereas in water with a PO_2 of 3 kPa (arterial PO_2 0.6 kPa), postprandial stimulation of protein synthesis and O_2 consumption tended to decrease although protein synthesis rates were tissue specific. An increase in environmental PO_2 (60 kPa, arterial PO2 10 kPa) resulted in an increase in protein synthesis compared with normoxic rates. In the hepatopancreas under normoxia there was a 30% rise in protein synthesis at 2 h post-feeding, a maximum at 5 h and a return to pre-feeding values within 48 h. The hepatopancreas responded to hypoxia by a down regulation of protein synthesis which is likely to be a

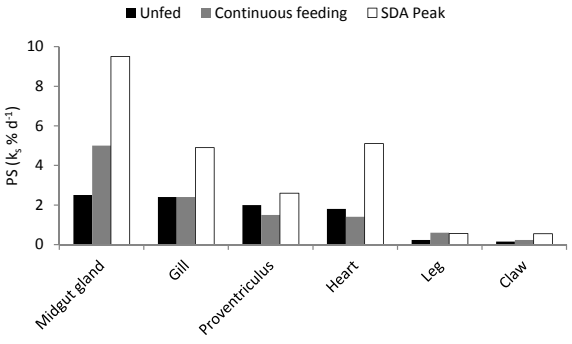

Figure 2. Protein synthesis (PS) expressed as a fractional rate (k_s, % d^{-1}) in different tissues of adult green crab in relation to feeding regime, data from [25].

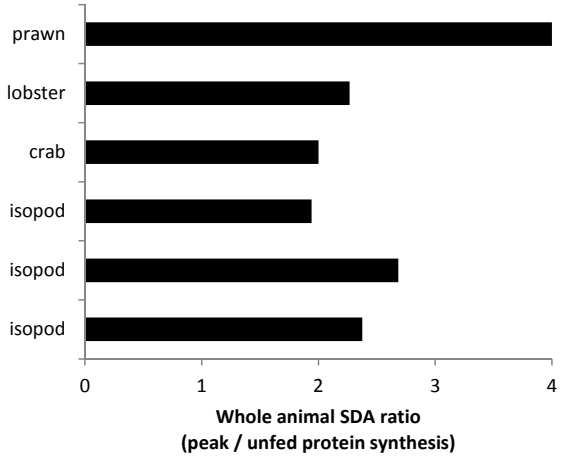

Figure 3. The whole-animal SDA-ratio (peak protein synthesis / unfed protein synthesis) for different crustaceans: prawn [31]; lobster [17]; crab [25]; and three isopods [30,36].

strategy to deal with reduced metabolic scope and increase the chance of survival. Crucian carp (*Carassius auratus*) can survive in anoxic water, this is in part due to the ability to significantly down regulate protein synthesis to reduce energy expenditure and oxygen requirements [42].

5. Diet composition

Three published studies have considered the effect of different diets on protein synthesis in crustaceans [26,27,31]. Three groups of Norwegian lobster (*Nephrops norvegicus*), either fed a laboratory diet, starved or freshly wild-caught, were compared in relation to protein synthesis as a correlate of nutritional status [26]. Based on protein synthesis in muscle and hepatopancreas the study confirmed that the wild-caught animals were of intermediate nutritional status between laboratory

fed and starved animals: tail muscle protein synthesis was higher than in starving animals and the same as in feeding animals; hepatopancreas protein synthesis was intermediate between feeding and starved animals. It was concluded that the wild-caught animals had fed less recently than the laboratory animals because the hepatopancreas protein synthesis was lower but they were feeding and growing (albeit at a slower rate) because the muscle protein synthesis was higher than in starved animals [26]. The research was of a preliminary nature and the individual variation was relatively large which meant some apparent differences were not statistically different. The effect on protein synthesis of the natural diet and of its different components would be of interest, the nutrient composition of different prey might have different effects on protein synthesis and on SRE.

The remaining two studies were laboratory based nutrition experiments in which differences in diet composition was the main variable being investigated. The influence of dietary protein was investigated in the brown tiger prawn (*Penaeus esculentus*) by feeding isoenergetic diets with 30, 40 or 50% protein to intermoult juveniles and measuring protein synthesis in the morning [27] when, presumably, they had not fed for at least 12 h. The highest protein growth was on the 40% protein diet [27] and this was achieved at the lowest fractional rate of protein synthesis and therefore at the lowest fractional rate of protein degradation (Figure 4a) and the highest SRE of 93% (Figure 4b). The SRE of 93% suggests a very high efficiency, this is because the use of a protein synthesis rate from unfed animals underestimated the daily rate of protein synthesis and therefore overestimated the proportion of protein retained and SRE (see below). Differences in growth and protein metabolism amongst the diets related to how well the diets met requirements for protein and energy. Recent recommended requirements for prawns vary between 21 and 27 mg digestible protein (DP) kJ^{-1} digestible energy (DE) for kuruma (*Marsupenaeus japonicus*) and tiger (*Penaeus monodon*) prawns [43]. Calculation of dietary protein energy suggests the 30, 40 and 50% protein diets had 14.8, 19.7 and 24.6 mg crude protein (CP) kJ^{-1} gross energy (GE), respectively. The NRC requirements show the 40 and 50% protein diets should have met the protein energy requirement and suggested the 50% protein diet might have performed better than it did. Thus, further research using prawns to investigate relationships between optimum dietary protein energy and efficiency of retaining protein would be highly informative.

The most comprehensive investigation of protein synthesis and amino acid metabolism in a crustacean was conducted on freshwater giant prawn (*Litopenaeus vannanmei*) post-larvae fed three formulated feeds with different protein ingredients: only marine proteins (fish, shrimp, squid meals); a 1:1 mix of the marine proteins and soy protein; only purified proteins (casein and caseinate) [30]. The purified feed performed less well than the two diets containing marine products, in relation to protein metabolism this was due to protein degradation being over 3 times higher and a SRE of 81% compared to 94% for the two marine protein feeds. The essential amino acid index (EAAI) was 0.95 and 0.85 for the marine and purified feeds, respectively, and the poorer amino acid balance of the purified feed explained the poorer performance. Dietary protein energy was slightly different between the feeds and ranged between 25 and 26.6 mg CP kJ^{-1} GE, for the marine and purified feeds, respectively. In addition to having a better EAA balance this analysis suggested the marine feeds were also closer to the optimum dietary protein energy of 23.9 mg CP kJ^{-1} DE for this species.

There is a strong relationship between dietary protein and energy and SRE (24 h) for salmonids that shows SRE increases towards the requirement of around 17-19 mg CP kJ^{-1} DE [2]. When the optimum protein is fed, salmonids are highly efficient at retaining it. When too much protein is fed, protein synthesis is

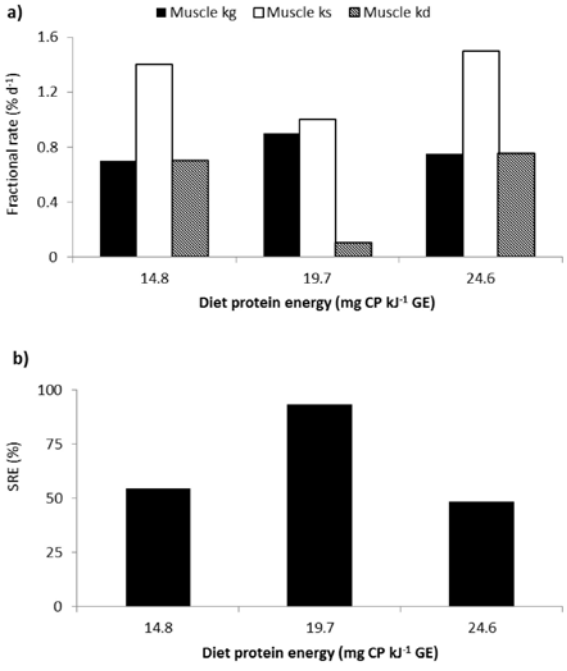

Figure 4. Protein metabolism of brown tiger prawn fed three feeds with different dietary protein energy (mg CP kJ^{-1} GE): a) fractional rates (% d^{-1}) of protein growth (k_g), synthesis (k_s) and degradation (k_d) and b) SRE, protein synthesis retention efficiency (%) [27].

stimulated to regulate amino acid fluxes but synthesised proteins are not retained as growth as efficiently. The SRE (24 h) values for prawns are above the salmonid line (Figure 5a) because there were measured for diets close to the optimum dietary protein energy where the prawns should retain protein most efficiently if they have a similar protein metabolism to the salmonids. The use of unfed protein synthesis values to calculate SRE provides an upper estimate of how well animals retain synthesised protein because protein synthesis measured at 24 h reflects a basal pre-feeding rate. Depending on the number of abiotic, biotic and specific nutritional factors some tissues have a pronounced post-prandial peak whereas others have more sustained plateau [2,39]. If unfed and peak rates of protein synthesis are measured, a daily protein synthesis value can be accurately calculated [39]; this was used to calculate daily SRE values of 38% for the marine protein feeds and 32% for the purified feed (Figure 5b).

The freshwater giant prawn study is a good example of how relative differences in protein growth and protein synthesis combine into a much larger difference in protein degradation. It illustrates that, sometimes, quite subtle differences in synthesis and growth underlie differences in overall protein metabolism and performance of diets [2]. There is considerable scope for further work around understanding protein metabolism and efficiencies in crustaceans. The lack of research in this area is surprising given the importance of prawn aquaculture [44].

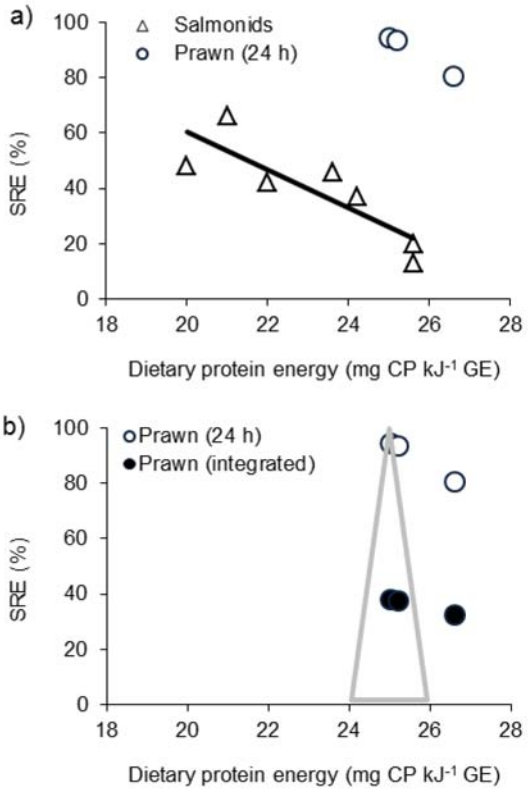

Figure 5. The relationship between dietary protein energy (mg CP kJ^{-1} GE) and SRE (protein synthesis retention efficiency, %): a) for salmonids, as adapted from Carter and Houlihan [2], and prawns [27] calculated from unfed (24 h) protein synthesis and b) for prawns calculated from unfed (24 h) protein synthesis or daily protein synthesis integrated over 24 h. The triangle base indicates the range of dietary protein energy requirements for prawn species [43].

6. Conclusions and future perspectives

Rates and efficiencies of protein synthesis are specific to species and developmental stage. As with other animals, protein synthesis is a major energy-demanding process in crustaceans and shown to be influenced by several abiotic and biotic factors. The effects of temperature in relation to species and latitude, and dissolved oxygen in relation to hypoxia and hyperoxia have been studied. Climate change effects, including elevated water temperature with the increased likelihood of hypoxia and ocean acidification, will significantly affect natural and farmed populations of crustaceans. Determining how climate change factors affect protein synthesis will be an important part of understanding how climate change will affect whole animal physiology. Of the many biotic factors that influence crustacean growth, protein synthesis has received little attention with only a few studies on the moult cycle and effects of hormones, feeding regimes, starvation and re-feeding, nutrition and diet composition. One area worthy of study is the high individual variation in growth rates, in fish differences in protein synthesis and turnover play an important part in explaining this [45].

Research on crustacean nutrition has progressed more slowly compared to similar research on fish partly because of the difficulty in experimentation on crustaceans caused by their “messy” feeding behavior and the need to sample at specific times in the moult cycle. The measurement of protein synthesis and protein turnover provides a subtle approach to assessing imbalances and deficiencies in dietary amino acid and energy [2]. Measuring protein synthesis has great potential for use in developing crustacean nutrition but has only been investigated in a few studies. For example, in prawns the retention of synthesized protein was higher at the optimum dietary protein content and when feeding dietary protein with an amino acid profile more similar to that of the prawns than not [31]. Similarly the most efficient feeding regimes in juvenile lobsters were demonstrated by the

highest efficiency of retaining synthesized protein. The relative importance of amino acids as energy sources in tissues in relation to growth and environmental factors such as temperature, salinity and anoxia needs further research.

The flooding-dose measurement of protein synthesis is relatively complex, time consuming and uses radioactive labels. Recommendations for the most effective use of the approach in aquaculture are: measure both basal and peak rates of protein synthesis to reflect metabolism before and following feeding; measure protein synthesis in key tissues, the hepatopancreas to reflect the digestive system and tail muscle to reflect growth; use basal and peak protein synthesis rates to calculate a daily rate for whole animals; integrate protein synthesis with growth; use the efficiency of retaining synthesized protein to understand and optimize parameters such as feeding regime and diet composition. In conclusion, crustacean protein metabolism requires further significant research to improve our knowledge of the unique physiology of crustaceans.

References

[1] Waterlow J.C., Protein Turnover, CABI Wallingford UK, 2006

[2] Carter C.G., Houlihan D.F., Protein synthesis, In: Wright P.A., Anderson P.M. (Eds.), Nitrogen Excretion, Fish Physiology Vol. 20, Academic Press San Diego, 2001

[3] Millward D.J., The nutritional regulation of muscle growth and protein turnover, Aquaculture, 1989, 79, 1-28

[4] Fraser K.P.P., Rogers A.D., Protein metabolism in marine animals: The underlying mechanism of growth, Ad. Mar. Biol., 2007, 52, 267-362

[5] Houlihan D.F., Carter C.G., McCarthy I.D., Protein turnover in animals, In: Wright P.J., Walsh P.A. (Eds.), Nitrogen and Excretion, CRC Press Boca Raton, 1995

[6] Carter C.G., Houlihan D.F., Brechin J., McCarthy I.D., The relationships between protein intake and protein accretion, synthesis and retention efficiency for individual grass carp, Ctenopharyngodon idella (Valenciennes), Can. J. Zool, 1993, 71, 392-400

[7] Houlihan D.F., Hall S.J., Gray C., Noble B.S., Growth rates and protein turnover in Atlantic cod, Gadus morhua., Can. J. Fish. Aquat. Sci., 1988, 45, 951-964

[8] Millward D.J., Rivers J., The nutritional role of indispensible amino acids and the metabolic basis for their requirements, Euro. J. Clin. Nutr, 1988, 42, 367-393

[9] Fraser K.P.P., Lyndon A.R., Houlihan D.F., Protein synthesis and growth in juvenile Atlantic halibut, Hippoglossus hippoglossus (L.): application of 15N stable isotope tracer, Aquacult. Res., 1998, 29, 289-298

[10] McCarthy I.D., Houlihan D.F., Carter C.G., Moutou K., Variation in individual food consumption rates of fish and its implications for the study of fish nutrition and physiology, P. Nutr. Soc., 1993, 52, 411-420

[11] Hawkins A.J.S., Protein turnover: a functional appraisal, Func. Ecol., 1991, 5, 222-233

[12] Houlihan D.F., Protein turnover in ectotherms and its relationships to energetics, Adv. Comp. Env. Physiol., 1991, 7, 1-43

[13] Houlihan D.F., Carter C.G., McCarthy I.D., Protein synthesis in fish., In: Hochachka P., Mommsen P. (Eds.), Biochemistry and Molecular Biology of Fishes, volume 4., Elsevier Science Amsterdam, 1995

[14] McCarthy I.D., Houlihan D.F., The effect of water temperature on protein metabolism in fish: the possible consequences for wild Atlantic salmon (Salmo salar L.) stocks in Europe as a result of global warming., In: Wood C.M., McDonald D.G. (Eds.), Global Warming: Implications for Freshwater and Marine Fish, Cambridge University Press, Cambridge, 1996

[15] Whiteley N.M., Robertson R.F., Meagor J., El Haj A.J., Taylor E.W., Protein synthesis and specific dynamic action in crustaceans: effects of temperature, Comp. Biochem. Physiol. A, 2001, 128, 595-606

[16] El Haj A.J., Houlihan D.F., In vitro and in vivo protein-synthesis rates in a crustacean muscle during the molt cycle, J. Exp. Biol., 1987, 127, 413-426

[17] Mente E., Houlihan D.F., Smith K., Growth, feeding frequency, protein turnover, and amino acid metabolism in European lobster Homarus gammarus L., J. Exp. Zool., 2001, 289, 419-432

[18] Gorell T.A., Gilbert L.I., Stimulation of protein and RNA synthesis in crayfish hepatopancreas by crustecdysone, Gen. Comp. Endo., 1969, 13, 308-310

[19] Gorell T.A., Gilbert L.I., Protein and RNA synthesis in premolt crayfish, Orconectes virilis, Zeitschrift Fur Vergleichende Physiologie, 1971, 73, 345-356

[20] Horst M.N., Association between chitin synthesis and protein-synthesis in the shrimp Penaeus vannamei, J. Crus. Biol., 1989, 9, 257-265
[21] Horst M.N., Concurrent protein-synthesis is required for in vivo chitin synthesis in postmolt blue crabs, J. Exp. Zool., 1990, 256, 242-254
[22] Paulson C.R., Skinner D.M., Effects of 20-hydroxyecdysone on protein-synthesis in tissues of the land crab Gecarcinus lateralis, J. Exp. Zool., 1991, 257, 70-79
[23] Garlick P.J., McNurlan M.A., Preedy V.R., A rapid and convenient technique for measuring the rate of protein synthesis in tissues by injection of 3H phenylalanine, Biochem J, 1980, 192, 719-723
[24] El Haj A.J., Clarke S.R., Harrison P., Chang E.S., In vivo muscle protein synthesis rates in the American lobster Homarus americanus during the moult cycle and in response to 20-hydroxyecdysone, J. Exp. Biol., 1996, 199, 579-585
[25] Houlihan D.F., Waring C.P., Mathers E., Gray C., Protein-synthesis and oxygen-consumption of the shore crab Carcinus maenas after a meal, Physiol. Zool., 1990, 63, 735-756
[26] Mente E., Carter C.G., Barnes R.S., Karapanagiotidis I.T., Protein synthesis in wild-caught Norway lobster (Nephrops norvegicus L.), J. Exp. Mar. Biol. Ecol., 2011, 409, 208-214
[27] Hewitt D.R., Response of protein turnover in the brown tiger prawn Penaeus esculentus to variation in dietary protein content, Comp. Biochem. Physiol. A, 1992, 103, 183-187
[28] Carter C., Houlihan D., Keissling A., Medale F., Jobling M., Physiological effects of feeding, In: Houlihan D., Boujard T., Jobling M. (Eds.), Food Intake in Fish, Blackwell Science Oxford, 2001
[29] Millward D.J., Garlick P.J., James W.P.T., Nnanyelugo D.O., Ryatt J.S., Relationship between protein synthesis and RNA content in skeletal muscle., Nature, 1973, 241, 204-205
[30] Robertson R.F., El Haj A.J., Clarke A., Peck L.S., Taylor E.W., The effects of temperature on metabolic rate and protein synthesis following a meal in the isopod Glyptonotus antarcticus Eights (1852), Polar Biol., 2001, 24, 677-686
[31] Mente E., Coutteau P., Houlihan D., Davidson I., Sorgeloos P., Protein turnover, amino acid profile and amino acid flux in juvenile shrimp Litopenaeus vannamei: effects of dietary protein source, J. Exp. Biol., 2002, 205, 3107-3122
[32] R.G., Growth in Crustacea-twenty years on, Hydrobiologia, 2001, 449, 11-122
[33] Moltschaniwskyj N.A., Carter C.G., Protein Synthesis, degradation, and retention: Mechanisms of indeterminate growth in cephalopods, Physiol. Biochem. Zool., 2010, 83, 997-1008
[34] Whiteley N.M., Taylor E.W., El Haj A.J., A comparison of the metabolic cost of protein synthesis in stenothermal and eurythermal isopod crustaceans, Am. J. Physiol., 1996, 271, R1295-R1303
[35] Mejean L., StrickerKrongrad A., Lluch A., Chronobiology, nutrition and metabolism, Pathol. Biol., 1996, 44, 603-609
[36] Robertson R.F., El Haj A.J., Clarke A., Taylor E.W., Effects of temperature on specific dynamic action and protein synthesis rates in the Baltic isopod crustacean, Saduria entomon, J. Exp. Mar. Biol. Ecol., 2001, 262, 113-129
[37] McCue M.D., Specific dynamic action: A century of investigation, Comp. Biochem. Physiol. A, 2006, 144, 381-394
[38] Katersky R.S., Carter C.G., The effect of temperature on post-prandial protein synthesis in juvenile barramundi, Lates calcarifer, Comp. Biochem. Physiol. A, 2010, 156, 529-536
[39] Carter C.G., Mente E., Barnes R.S., Nengas I., Protein synthesis in gilthead sea bream: response to partial fishmeal replacement, Br. J. Nutr., 2012, 108, 2190-2197
[40] Whiteley N., Faulkner L.S., Temperature influences whole-animal rates of metabolism but not protein synthesis in a temperate intertidal isopod, Physiol. Biochem. Zool., 2005, 78, 227-238
[41] Mente E., Legeay A., Houlihan D.F., Massabuau J.C., Influence of oxygen partial pressures on protein synthesis in feeding crabs, Am. J. Physiol., 2003, 284, R500-R510
[42] Smith R.W., Houlihan D.F., Nilsson G.E., Brechin J.G., Tissue-specific changes in protein synthesis rates in vivo during anoxia in crucian carp, Am. J. Physiol., 1996, 271, R897-R904
[43] National Research Council, Nutrient Requirements of Fish and Shrimp, The National Academies Press, Washington, D.C., 2011
[44] Intanai I., Taylor E.W., Whiteley N.M., Effects of salinity on rates of protein synthesis and oxygen uptake in the post-larvae and juveniles of the tropical prawn Macrobrachium rosenbergii (de Man), Comp. Biochem. Physiol. A, 2009, 152, 372-378
[45] McCarthy I.D., Houlihan D.F., Carter C.G., Individual variation in protein turnover and growth efficiency in rainbow trout, Oncorhynchus mykiss (Walbaum), Proc. Roy. Soc. Lond. B., 1994, 257, 141-147