RESEARCH PAPER

Culture conditions affect the nutritional value of the copepod *Acartia tonsa*

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حالات الإستزراع وتأثيرها على القيمة الغذائية للكوبابودا *Acartia tonsa*

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ABSTRACT. Live feed are still necessary for the rearing of larval stages of several fish species, especially marine ones. Compared to *Artemia*, copepods are of superior quality. This is based on a suite of traits like size, movement, and nutritional value. Copepods are for example usually high in protein and fatty acids. Essential fatty acid profiles reflect to a large degree the fatty acid supply, which provides the opportunity to manipulate fatty acid profiles of, amongst others, copepods. By manipulating nutrient supply of the algae *Rhodomonas salina* we were able to double essential fatty acid concentrations in naupliar and copepodit life stages of the copepod *Acartia tonsa*. However, this lead to growth depression rather than to increased growth rates in a series of consumer species, including larval fish. The reason for the growth depression is likely to be mineral deficiencies occurring along with the nutrient manipulation of the algae.

Keywords: copepod, nutritional value, live feed, aquaculture, *Acartia*

المستخلص: تبقى المغذيات الحية ضرورية لتربية اليرقات للعديد من الأسماك وخصوصا البحرية منها. وتعتبر الكوبابودا ذات جودة عالية مقارنة مع الأرتيميا. ويستند هذا على مجموعة من الصفات مثل الحجم، والحركة، والقيمة الغذائية. وتحتوي الكوبابودا على سبيل المثال على نسبة عالية من البروتين والأحماض الدهنية. وتعكس ملامح الأحماض الدهنية الأساسية إلى حد كبير إمدادات الأحماض الدهنية، والتي تتيح الفرصة لمعالجة ملامح الأحماض الدهنية من بين أمور أخرى. وبمعالجة التزويد بالمواد الغذائية الطحلبية (رودوماس سلينا) تمكنا من مضاعفة تركيز الأحماض الدهنية الأساسية في مراحل حياة الكوبابودا . إلا أن ذلك أدى إلى القصور في النمو بدل الزيادة في معدلات النمو في سلسلة من الأنواع المستهلكة بما في ذلك اليرقات. وقد يكون النقص في المعادن سببا مرجحا لذلك، نتيجة لمعالجة المغذيات الخاصة بالطحالب.

الكلمات المفتاحية: غراء البتيدات، السيلوكسانات، الخاصة بالطحالب.
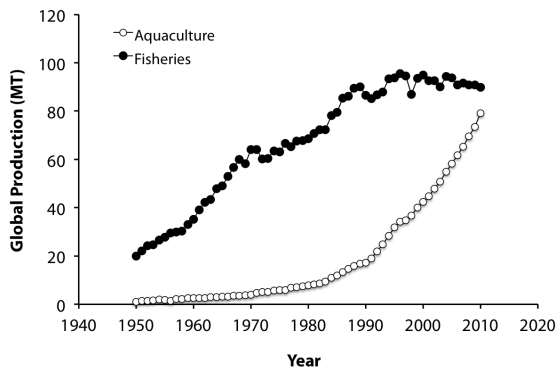
Introduction

The global yield of the capture fisheries has been stagnating at around 85 million metric tonnes per year since the mid 1980ies. Since more or less the same time, aquaculture production is on an exponential rise, replacing the missing growth in capture fisheries production (Fig. 1). The vast majority of the 80 million tons of aquaculture production in 2010 was made up by seaweeds and cyprinid fishes (20 and 25 million tonnes respectively) (Fao). However, focussing on the value per unit the picture completely changes and next to the high value products such as abalone, shrimps and sturgeons with values between 20,000 and 50,000 US$ tonne-1 we find species such as groupers, soles and pompanos in the same price range. The main difference between the first and the latter group is that the first is relatively

easy to culture and the price is based on slow growth and relatively low area based biomass production. The latter group of groupers, soles and jacks have in common that they are sought after food fish with dwindling stocks and that they are not easy to grow, reproduce and wean in captivity. One of the bottlenecks for instance in grouper culture is the larval weaning (Sugama *et al.* 2012), and they, like many other species, rely on live feeds (Lavens and Sorgeloos 1996; Lavens *et al.* 1994). This is mirrored by the still increasing number of publications on the use of live feeds in aquaculture (Fig. 2). The typical succession of live feeds for very small, gape limited larvae is to start with ss-type or s-type Brachionus spp., followed by larger Brachionus spp. strains and eventually Artemia spp. larval stages (Lavens and Sorgeloos 1996; Lavens *et al.* 1994). *Artemia* and *Brachionus* species are not always the most suitable first feeds for marine fish larvae due to inappropriate size (Pepin and Penney 1997; Van Der Meeren 1991), their swimming behaviour, which makes them less susceptible to predation (Buskey *et al.* 1993; Von Herbing and Gallagher 2000), and they are suspected of being of insufficient nutritional quality (Støttrup and Norsker 1997). Calanoid copepods as live feed have been shown to improve growth and survival in groupers (Doi *et al.* 1997), and several other species (Stottrup

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Figure 1. Global wild fish capture and aquaculture production in million tonnes, 1950–2010. Data source: FAO (Fao).
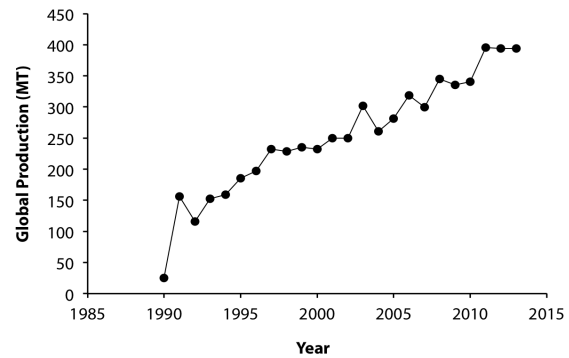
Figure 2. Number of publications retrieved from Web of Science using the search words 'aquaculture' and 'live feeds'

2000), and larval grouper actively select for copepod nauplii over rotifers (Toledo *et al.* 2004). Similar selectivity patterns have been reported for a suite of marine fish larvae (Monteleone and Peterson 1986; Stoecker and Govoni 1984).

Food quality can be expressed in many ways, such as through the concentration of polyunsaturated fatty acids (Paulsen *et al.* 2014; Paulsen *et al.* 2013), sterols (Lee 2001), amino acids (Awaiss *et al.* 1992) or the elemental composition (Malzahn *et al.* 2007; Shao *et al.* 2008) of the food item. All of the latter play a major role in fish nutrition (Lavens *et al.* 1994), and it is crucial to find the food organisms which suits the demand best. It is very likely that marine copepods are a good match for the nutritional demands of larval fish as they are the main prey items in nature, hence an adaptation of the needs of larval fish to the main prey in supply can be assumed. Wild zooplankton has been successfully used for larval rearing (Otterlei *et al.* 1999) with growth rates superior compared to *Artemia* larvae. However, availability of natural zooplankton is a problem, as zooplankton size spectrum (Beaugrand *et al.* 2002; Greve *et al.* 2004), density (Beaugrand *et al.* 2003) and species composition (Beaugrand *et al.* 2002; Greve *et al.* 2004) are permanently changing. Even if the supply of natural

zooplankton was reliable, the nutritional value of copepods varies with species (Gismervik 1997a; Gismervik 1997b), life stage (Villar-Argaiz *et al.* 2002; Villar-Argaiz and Sterner 2002) and season (Villar-Argaiz *et al.* 2002; Villar-Argaiz and Sterner 2002) and is hence again an unreliable source of live feeds. Consequently, in-house life feed production in the form of copepods seems to be a reliable way to fulfil the nutritional demand of early life stages of fish until they can be weaned on more easily accessible live feeds like *Brachionus* and *Artemia* or even formulated diets.

This paper aims to sum up a series of experiments carried out to determine how copepod culture conditions can be manipulated to produce copepods of an elemental and biochemical composition of choice.

Materials and methods

We conducted a series of experiments in which copepods were fed on phytoplankton which in turn was grown in nutrient manipulated growth media. The data we show here are representative for the nutrient treatments and can easily be reproduced. The phytoplankter R. salina was reared either under nutrient replete conditions using the f/2 medium following Guillard and Ryter

Table 1. Fatty acid concentrations and elemental ratios of phytoplankton grown on nutrient replete (f/2) and nutrient depleted (-P and –N) media.

Measure	f/2	+/- sd	-N	+/- sd	-P	+/- sd
sum unsaturated FA (µg*µgC-1)	0.035	0.022	0.066	0.032	0.105	0.079
total FA (µg*µgC-1)	0.058	0.023	0.145	0.056	0.146	0.090
20:5n3 (EPA) (µg*µgC-1)	0.009	0.007	0.011	0.006	0.016	0.011
22:6n3 (DHA) (µg*µgC-1)	0.007	0.005	0.017	0.017	0.024	0.039
C:N (mol*mol-1)	7.59	0.66	10.14	2.65	8.03	1.72
C:P (mol*mol-1)	231.13	86.71	173.12	84.60	579.24	108.17
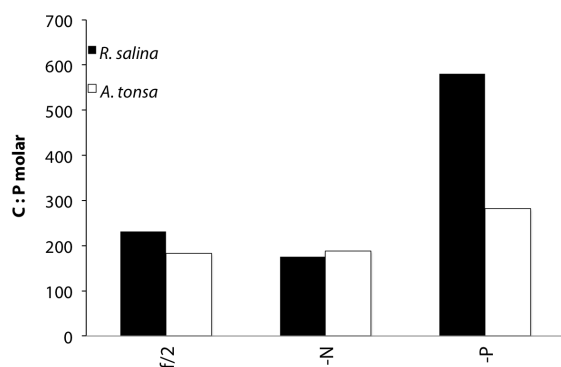
Figure 3. C:P ratios of R. salina grown on nutrient replete (f/2) and nutrient depleted (-P and –N) conditions and *A. tonsa* reared on these algae.
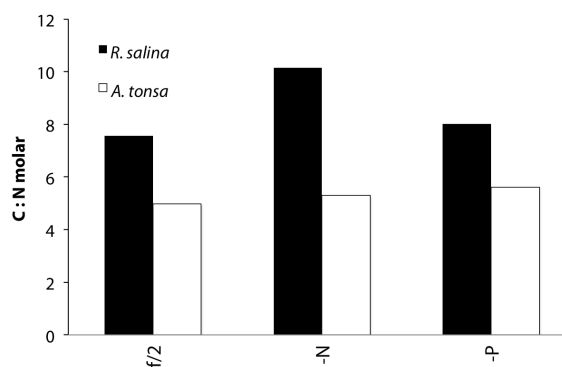
Figure 4. C:N ratios of R. salina grown on nutrient replete (f/2) and nutrient depleted (-P and –N) conditions and *A. tonsa* reared on these algae.

Table 2. Statistical information (one way Anova followed by Tukeys HSD test for unequal n) on various fatty acid measures (µg FA*µg carbon-1) from phytoplankton reared on nutrient replete (f/2) and nutrient depleted (-P and –N) conditions.

Measure		SS	df	F	p	f/2 vs -P	f/2 vs -N	-N vs -P
Total Fatty acids	Intercept	0.741	1	192.020	>0.05	<	<	n.s.
	Treatment	0.096	2	12.449	>0.05			
	Error	0.201	52					
Unsaturated FA	Intercept	0.258	1	101.979	>0.05	n.s.	<	n.s.
	Treatment	0.046	2	9.119	>0.05			
	Error	0.131	52					
20:5n3 (EPA)	Intercept	0.008	1	117.231	>0.05	n.s.	<	n.s.
	Treatment	0.001	2	4.047	>0.05			
	Error	0.003	52					
22:6n3 (DHA)	Intercept	0.013	1	22.242	>0.05	n.s.	n.s.	n.s.
	Treatment	0.003	2	2.283	>0.05			
	Error	0.031	52					

(1962) or under nitrogen or phosphorus limitation. The phosphorus limited treatment was realized by adding all f/2 ingredients but phosphorus to sterile filtered natural seawater. This means that the phytoplankton could only use the phosphorus which was available in the seawater at the moment of filtration. Adding all of the other macro- and micronutrients assured a Liebig/Sprengel type phosphorus limitation (Sprengel 1839). Nitrogen limitation was realized by adding 20% of the N usually added to the f/2 medium. The addition of some nitrogen was necessary to produce enough N-limited phytoplankton to suit the experimental needs. In order to ensure constant algal quality, a new batch of algae was set up every day and cultured well into the stationary phase for the N and P limited cultures. F/2 algae were always harvested in the exponential growth phase (Malzahn *et al.* 2007; Malzahn and Boersma 2012; Malzahn *et al.* 2010).

The calanoid copepod *Acartia tonsa* was then fed on these nutrient manipulated phytoplankton for a pre-defined period, after which the copepods were analysed for elemental and biochemical composition as well as their developmental rates. Carbon and nitrogen were analysed by means of an elemental analyser. Phospho-

rus was analysed as orthophosphate after acidic oxidative hydrolysis with 5% H_2SO_4 (Grasshoff *et al.* 1999). Fatty acids were analysed as fatty acid methyl esters and gas chromatography (for details see Malzahn *et al.* 2007; Malzahn and Boersma 2012; Malzahn *et al.* 2010; Schoo *et al.* 2013a; Schoo *et al.* 2013b). Developmental rates were calculated by dividing the mean developmental stage by the age of the animals. Due to the isochronal growth of *A. tonsa*, which means that all developmental stages are equally long (Berggreen *et al.* 1988; Miller *et al.* 1977) under constant growth conditions, it was not necessary to apply a weighing factor for certain developmental stages.

Results and Discussion

The different nutrient limitations the algae were subjected to resulted in significant differences in carbon-to-nutrient ratios and fatty acid profiles. Phytoplankton significantly varied in their C:N and C:P ratios with respect to the nutrient treatment (Fig. 3, Fig. 4 and Table 1). The concentrations of the limiting elements were always lower than the non-limiting elements, pointing on

Table 3. Statistical information (one way Anova followed by Tukeys HSD test for unequal n) on various fatty acid measures (expressed as %of total FA) from phytoplankton reared on nutrient replete (f/2) and nutrient depleted (-P and –N) conditions.

Measure		SS	df	F	p	f/2 vs -P	f/2 vs -N	-N vs -P
% sum unsaturated FA	Intercept	143652.115	1	541.516	>0.05	n.s.	n.s.	n.s.
	Treatment	1388.279	2	2.617	0.08			
	Error	13794.431	52					
% 20:5n3 (EPA)	Intercept	5270.416	1	243.938	>0.05	>	>	n.s.
	Treatment	269.813	2	6.244	>0.05			
	Error	1123.487	52					
% 22:6n3 (DHA)	Intercept	6571.439	1	61.112	>0.05	n.s.	n.s.	n.s.
	Treatment	62.834	2	0.292	0.75			
	Error	5591.643	52					

the non-homoeostatic nature of phytoplankton growth (Droop 1973; Droop 1974). The concentration of fatty acids also varied significantly with nutrient limitation, showing generally higher fatty acids concentrations when grown under nutrient limitation (Table 1 & Table 2). Similar, as well as opposing patterns, have been reported for other phytoplankton species (Reitan *et al.* 1997). The majority of variability in fatty acid concentrations in general seems to be introduced by taxonomic group. However, the variance due to culture conditions can be substantial as well (Reitan *et al.* 1994). This species specific behaviour of altering fatty acid production with nutrient supply enables the keen aquaculturist to tailor single species cultures or even mixes of different phytoplankton species to suit the needs of live food and subsequently the larval fish. Considering fatty acids not as concentrations but as percentage of total fatty acids revealed a different picture (Table 3). Here we found fewer differences between the treatments, which indicate that fatty acids production might vary in quantity, but that the variation in relative proportions is less pronounced.

When copepods were fed such manipulated phytoplankton we found the same pattern of increased elemental ratios and fatty acid enriched copepods as when they were fed on phosphorus limited phytoplankton

(Figure 3 & Figure 4, Table 4 & Table 5). Looking at the relative contribution of fatty acids to the total fatty acid pool we found no differences between the treatments (Table 6), which points to the conservative nature of the propagation of fatty acids from one trophic level to the next (reviewed in Dalsgaard *et al.* 2003). However, not only did the fatty acid concentrations change in copepods when fed on nutrient limited phytoplankton, but so did the elemental composition. We found increased C:P ratios in copepods reared on phosphorus limited phytoplankton in several experiments (Malzahn *et al.* 2007; Malzahn and Boersma 2012; Schoo *et al.* 2010; Schoo *et al.* 2012; Schoo *et al.* 2013a). Consumers have a dome-shaped growth response to food carbon to phosphorus ratios, growth being carbon (energy) limited on the low C:P side and phosphorus limited on the high C:P side (Boersma and Elser 2006). High C:P ratios create problems because of the excess carbon which has to be taken up with every unit of phosphorus. The handling of the excess carbon seems to create costs high enough to significantly depress consumers' growth. The phytoplankton in our experiments showed superior fatty acid profiles and inferior C:P ratios under phosphorus limitation, the former known to accelerate growth (Dalsgaard *et al.* 2003; Engstrom-Ost *et al.* 2005; Izquierdo *et al.* 2000), the latter known to depress growth (Sterner

Table 4. Fatty acid concentrations and elemental ratios of copepods grown on nutrient replete (f/2) and nutrient depleted (-P and –N) phytoplankton.

Measure	f/2	+/- sd	-N	+/- sd	-P	+/- sd
sum unsaturated FA ($\mu g*\mu gC^{-1}$)	0.077	0.024	0.119	0.053	0.136	0.058
total FA ($\mu g*\mu gC^{-1}$)	0.013	0.006	0.024	0.011	0.036	0.016
20:5n3 (EPA) ($\mu g*\mu gC^{-1}$)	0.002	0.003	0.003	0.004	0.006	0.003
22:6n3 (DHA) ($\mu g*\mu gC^{-1}$)	0.003	0.003	0.013	0.012	0.013	0.008
C:N ($mol*mol^{-1}$)	4.989	0.127	5.303	0.628	5.617	0.580
C:P ($mol*mol^{-1}$)	180.912	39.585	186.018	65.281	280.917	96.898

Table 5. Statistical information (one way Anova followed by Tukeys HSD test for unequal n) on various fatty acid measures (µg FA*µg carbon[-1)]) from copepods reared on nutrient replete (f/2) and nutrient depleted (-P and –N) phytoplankton.

Measure		SS	df	F	p	f/2 vs -P	f/2 vs -N	-N vs -P
tot. FA	Intercept	0.367	1	162.96	>0.05	n.s.	<	n.s.
	Treatment	0.019	2	4.11	>0.05			
	Error	0.061	27					
sum unsaturated FA	Intercept	0.018	1	125.44	>0.05	n.s.	<	n.s.
	Treatment	0.003	2	9.52	>0.05			
	Error	0.004	27					
20:5n3 (EPA)	Intercept	0.000	1	39.92	>0.05	n.s.	<	n.s.
	Treatment	0.000	2	3.53	>0.05			
	Error	0.000	27					
22:6n3 (DHA)	Intercept	0.003	1	35.42	>0.05	<	<	n.s.
	Treatment	0.001	2	4.20	>0.05			
	Error	0.002	27					

Table 6. Statistical information (one way Anova followed by Tukeys HSD test for unequal n) on various fatty acid measures (expressed as % of total FA) from copepods reared on nutrient replete (f/2) and nutrient depleted (-P and –N) phytoplankton.

Measure		SS	df	F	p	f/2 vs -P	f/2 vs -N	-N vs -P
% sum unsaturated FA	Intercept	26614.51	1	360.38	>0.05	n.s.	<	n.s.
	Treatment	1487.43	2	10.07	>0.05			
	Error	1920.13	26					
% 20:5n3 (EPA)	Intercept	259.71	1	59.66	>0.05	n.s.	n.s.	n.s.
	Treatment	12.12	2	1.39	0.267			
	Error	113.18	26					
% 22:6n3 (DHA)	Intercept	1698.21	1	44.32	>0.05	n.s.	n.s.	n.s.
	Treatment	223.30	2	2.91	0.072			
	Error	996.19	26					

1993; Sterner *et al.* 1993; Sterner and Hessen 1994). The question arising from this is distinguishing which factor is more important for e.g. larval fish growth. The unique biochemical composition of the phytoplankter *R. salina* and the relatively strong conservation of fatty acids as well as C:P signals in the copepod *A. tonsa* allowed us to test this. In all of the above mentioned experiments high copepod C:P resulted in reduced growth rates in larval herring (Malzahn *et al.* 2007), gelatinous zooplankton (Schoo *et al.* 2010) and larval European lobster (Schoo *et al.* 2012; Schoo *et al.* 2013a). This finding leads to the conclusion that mineral requirements have to be fulfilled first before biochemical requirements like fatty acids can promote growth. Consequently, not one single measure should be the focus when optimizing live feeds for aquaculture, but a more holistic approach will lead to better results.

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