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Diatom production in the marine environment: implications for larval fish growth and condition

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To test the effects of diatom production on larval fish growth and condition, laboratory experiments were performed with larval North Sea cod reared on different algal food chains. These food chains were based on cultures of (a) the diatoms *Skeletonema costatum* and *Thalassiosira weissflogii*; (b) the dinoflagellate *Heterocapsa triquetra*; (c) the flagellate *Rhodomonas baltica*; (d) a diet composed of both *Skeletonema* and *Heterocapsa* food chains (1:1), and (e) a starvation group. These algae were fed to cultures of adult *Acartia tonsa*. Copepod eggs were collected, hatched, and the N1 nauplii ($200\,l^{-1}$) were fed to post-yolk-sac larval cod. Results indicate that larval growth rates are significantly influenced by the content of essential fatty acids of the algal food source: growth rates were positively correlated with the content of DHA (C22:6ω3) and negatively with EPA (C20:5ω3). The ratio of ω3/ω6 fatty acids in the algal source had no significant effect. The highest and lowest growth rates were observed in food chains based on *H. triquetra* and *T. weissflogii,* respectively (means for days 14–16 of 4.0 and −4.7). The mixed diatom/dinoflagellate diet resulted in intermediate growth rates and condition. Regressions of growth rates against EPA and DHA content indicated no inhibitory effect of diatom production on growth in larval cod.

Keywords: diatom, essential fatty acids, cod, larval growth, RNA/DNA.

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Introduction

Fish yield in the marine environment is ultimately dependent upon the amount of primary production and the efficiency of transfer to higher trophic levels. Two types of ecosystems with large differences in transfer efficiency and amount of primary production have been identified. The first type is based on the injection of nitrate from the deep layer into a nutrient-replete water column resulting in "new" primary production (Dugdale and Goering, 1967). These systems are representative of the classical short food chain based on the transfer of diatom production to large copepods and finally to fish, and result in the major pelagic fish stocks of the world. The fisheries on these are typically situated in the major upwelling regions of the oceans (Cushing, 1971). These systems are assumed to be efficient in the transfer of primary production to higher trophic levels owing to the relatively direct transfer of biomass (Parsons *et al*., 1984).

The second type is based on the microbial loop community, where the majority of primary production is the result of recycling of nutrients by micro-organisms (Azam *et al*., 1983). These communities exist primarily in stable oligotrophic water columns, where bacteria, pico- and nanoplankton, heterotrophic flagellates, and dinoflagellate species dominate primary production. Owing to the large number of trophic interactions involved, and the loss of energy in the transport between levels, such ecosystems are relatively inefficient and are typically characterized by low fish yield (e.g. tropical fisheries).

Several processes have been identified that affect the efficiency of transfer of primary production to higher trophic levels. For instance, the generation times

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of planktonic organisms vary greatly between these ecosystems. A rapid and efficient transfer of production occurs in the microbial loop system as primary producers and their predators have generation times on the order of days, thereby allowing a rapid increase of predator populations in response to prey blooms. In the case of the classical linear food chain, the generation times of the primary producers are in the order of days, while those of their predators are in the order of weeks to months (Kiørboe, 1991). Here, except in specific oceanographic regions (i.e. frontal circulation; Franks and Chen, 1996), herbivore populations are unable to respond to rapid increases in phytoplankton biomass and much of the primary production is lost to the benthos (Kiørboe, 1991; Josefson and Conley, 1997).

Also, the suitability of prey species for growth and reproduction of organisms at higher tropic levels may modify the transfer of biomass from primary producers. This variation in suitability results predominantly from two mechanisms. The first is the presence of toxic or inhibitory components produced by the prey species (Ianora *et al*., 1995; Shaw *et al*., 1997). These substances result in a modification of feeding behaviour and in avoidance of specific food particles (Shaw *et al*., 1995) as well as having a potential impact on reproductive success via abnormal embryonic and post-hatch development (Poulet *et al*., 1995). A second mechanism influencing suitability is the dietary composition of the prey; in particular, inadequate levels of the essential fatty acids (EFA) eicosapentaenoic acid (EPA; C20:5ω3) and docosahexaenoic acid (DHA;C22:6ω3). Deficiencies of these EFA reduce growth rates (Langdon and Waldock, 1981; Thompson and Harrison, 1992) and reproductive success in marine crustaceans (Xu *et al*., 1994; Jónasdóttir and Kiørboe, 1996; Pond *et al*., 1996). In marine fish species, an EFA-deficient diet has been observed to result in a reduction of total egg production, embryonic development, and hatch success (Watanabe, 1982). After hatch, EFA composition of food affected growth rates in larval and juvenile fish (Watanabe 1982; Koven *et al*., 1993) as well as the ability to feed (Bell *et al*., 1995).

We examine potential influences of variations in the EFA composition of mono-species algal blooms on growth of post-yolk-sac larval cod. In particular, we investigate the impact of the diatom *Skeletonema costatum* previously implicated in reduced reproductive success and mortality of the copepod *Temora stylifera* (Ianora *et al*., 1995).

Materials and methods

Rearing experiments

Experiments were carried out in March 1992, 1995 using pooled samples of eggs from North Sea cod that were fertilized as outlined in Munk and Rosenthal (1983). After hatching, and upon absorption of the yolk sac by approximately 80% of the larvae, they were transferred to cylindrical experimental rearing tanks containing 172 l of seawater (27 ppt). Temperature during incubation and later in the experimental tanks was maintained at 8.4 and 8.2°C, respectively ($\pm$ 0.1°C). Filtered seawater was continuously added to the tanks and drained through a stand pipe containing a 20-μm mesh nitex filter. Light conditions were varied from 0 to 1000–1300 Lux (at the water surface) as previously described by Støttrup and Munk (1983).

Four cultures of adult *Acartia tonsa* were fed ad libitum on mono-cultures of *Skeletonema costatum*, *Thalassiosira weissflogii*, *Heterocapsa triquetra*, and *Rhodomonas baltica*. Semi-continuous cultures of these algae were maintained under constant light and temperature (16.0 $\pm$ 0.2°C) with silica added to the growth media (B1) for the diatom cultures. Phytoplankton samples for determination of fatty acid composition were obtained randomly throughout the study. Phytoplankton samples were filtered onto pre-combusted GF-C filters and stored at -80°C under nitrogen gas prior to analysis.

From the four adult *Acartia tonsa* cultures, eggs were collected, enumerated, and hatched following procedures outlined by Stottrup and Munk (1983) and Stottrup *et al*. (1986). The resultant nauplii (stages N1 and N2) were fed to the cod larvae in the experimental cylinders at a density of 200 l^{-1}, so that each larval population was maintained on nauplii with a singular food chain history. Furthermore, one cod population was fed with a mixed diet of nauplii reared on *Skeletonema* and *Heterocapsa* (1:1) and the sixth and last one served as starvation control.

From each experimental population, 20 cod larvae were removed daily at the beginning of the light cycle. Larvae were examined microscopically for evidence of feeding, measured (total length) and then individually preserved under nitrogen gas and stored at -80°C until analysis. After the samples were taken from the tanks, nauplii abundance was determined at three depths in each tank (excluding starvation trial) and their concentration was raised to 200 l^{-1}.

Lipid analysis

Methyl esters of the fatty acids were prepared by saponification and methylation with methanolic boron trifluoride (Whyte, 1988). Individual fatty acid methyl esters were identified on a Hewlett Packard 5890 Gas Chromatograph equipped with a split/splitless injector and an auto-sampler. The Whyte (1988) procedure was modified to include the injection of the internal standards Heptadecanoic acid methyl ester (C17:0) and cholestane (Sigma) prior to the methanolysis of the

lipids from samples. Fatty acid methyl esters were identified by comparison with retention times of methyl esters obtained from Sigma and Larodan using techniques outlined in St. John and Lund (1996).

Growth and nucleic acid analysis

Prior to the determination of RNA and DNA contents, larvae were measured (standard length) and after freeze drying for 24 h at −52°C (Christ alpha 1.4) weights were determined on a Sartorius Microbalance. Growth rate (G) was calculated as percentage change in body weight (% d^{-1}) using the formula given in Ricker (1979):

$$G=(\ln w_2 - \ln w_1)/(t_2 - t_1) \times 100 \quad (1)$$

where w_1 is initial weight, w_2 is final weight, and t_1 and t_2 are the initial and final days of the experimental period. RNA and DNA content were determined according to Clemmesen (1993) with slight modifications using a specific nucleic acid fluorescent dye, Ethidium Bromide (EB).

To convert the measured RNA/DNA ratios to protein growth rates, a model by Buckley (1984) using ambient water temperature was used. Based on intercalibration experiments using Buckley's (1979) and Clemmesen's (1993) technique, a conversion factor was calculated for RNA and DNA content and their ratios as determined with the two different methods. The measured RNA/DNA ratios were thus converted (factor 1.3) to values that would have resulted from Buckley's method. These corrected values were then used for the calculation of protein growth rates according to (Buckley, 1984):

$$Gpi=0.93\ T+4.75\ RNA/DNA - 18.18 \quad (2)$$

where Gpi is the daily rate of protein growth and T is the ambient temperature.

Results

The changes in larval dry weight for the six alternative food types are given in Figure 1. Analyses of the effects of diet composition on larval growth were performed for the last 3 d of the period, thereby avoiding potential contributions of the yolk sac to growth (Table 1). The greatest increases in % dry weight per day (12.5% d^{-1}) was observed for the *Heterocapsa*-based diet, followed by *Rhodomonas* and *Thalassiosira* with the lowest (1.4%) observed in the *Skeletonema*-based feeding group. The mixed-diet group had an intermediate increase. Starved larvae showed no increase up to day 12, after which no larvae survived.

The difference in RNA content per larva (as a measure of the protein biosynthesis machinery) for the different experimental groups is shown in Figure 2. The

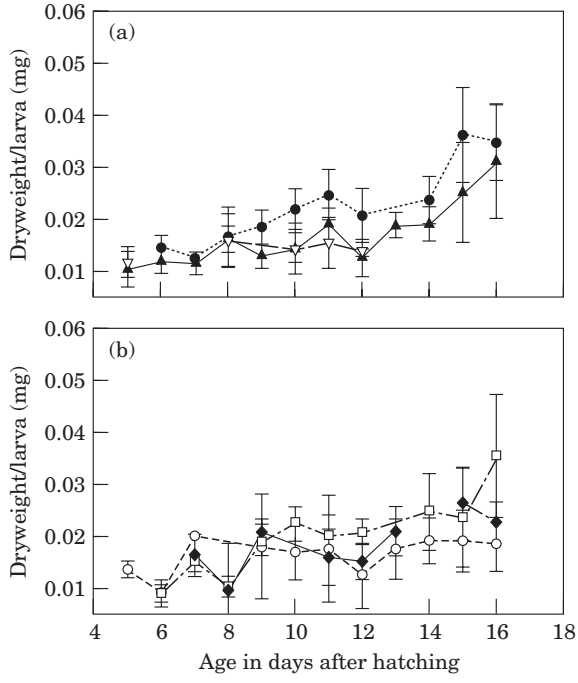

Figure 1. Relationship between mean dry weight (mg) and age of cod larvae: (a) starved larvae (inverted, open triangles) and larvae fed indirectly, through *Acartia* nauplii, on *Heterocapsa* (closed triangles), and *Rhodomonas* (closed circles) and (b) on *Skeletonema* (open circles), *Thalassiosira* (open squares), and a mixed 1:1 diet of *Skeletonema* and *Heterocapsa* (closed diamonds) (means and standard deviations of 5–10 individuals).

starved control group showed a decrease in the amount of RNA from day 5 to day 12. The *Skeletonema*-based group showed a slight temporal decrease after day 10, resulting in an overall negative RNA rate of change of −1.9% d^{-1} between day 10 and day 16 (Table 1). All other groups showed a positive rate of change, with the highest increase found in the *Heterocapsa*-based group (14.1% d^{-1}).

The highest RNA/DNA ratios were also found in the larval group fed a *Heterocapsa*-based diet [Figure 3(a)]. A significant change was observed between day 11 and day 12, when the mean ratios rose from 1.6 to 4.5. Between day 12 and day 16 the ratios fluctuated around a mean value of 4.1. The RNA/DNA ratios of the other feeding groups had lower values between day 12 and day 16 (2.7 for the mixed algae group and 2.0 for the *Skeletonema*-, *Thalassiosira*-, and *Rhodomonas*-based food chains). The starvation control showed a decrease from 1.4 (day 5) to 0.9 (day 12).

Based on the mean RNA/DNA ratios between days 14–16 and the model by Buckley (1984), the protein growth rates (Gpi) for the different feeding groups were calculated. The *Heterocapsa*-based feeding group had a positive protein growth rate of 4.0% d^{-1} (Table 1). The

Table 1. Comparison of changes in dry weight (mean for days 10–16) and RNA content (mean for days 10–16) and Gpi (mean and standard deviation for days 14–16; number of observations = 15 in all cases) of cod larvae reared indirectly on different algae (all values in % d^{-1}). Significance ($p<0.05$) of differences in Gpi was tested by pairwise multiple comparison procedures (Dunn's Method Sigmastat version 2.03).

Acartia diet	Dry weight	RNA	Gpi Mean	Gpi s.d.	Different from
Skeletonema	1.4	−1.9	−1.7	2.9	*Thalassiosira*, *Rhodomonas*
Thalassiosira	7.5	7.7	−4.7	2.4	*Heterocapsa*, mixed
Rhodomonas	7.5	4.9	−3.3	1.9	*Heterocapsa*
Heterocapsa	12.5	14.1	4.0	5.1	*Thalassiosira*, *Rhodomonas*
Mixed	7.0	7.5	−0.3	2.0	*Thalassiosira*

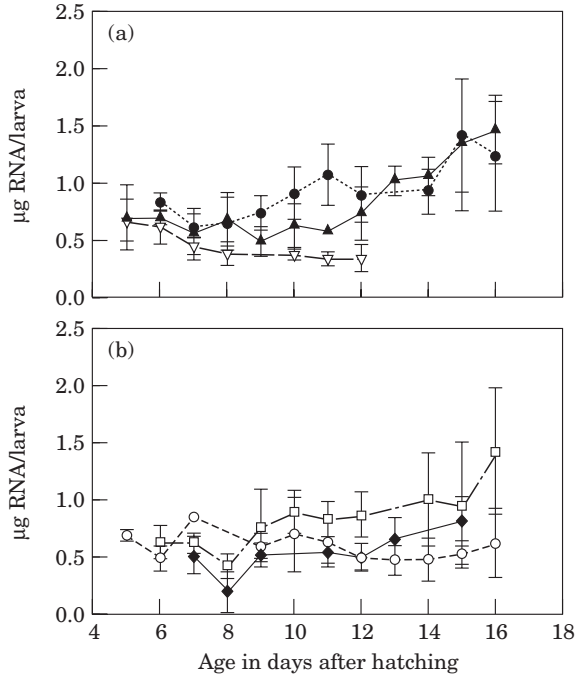

Figure 2. Relationship between RNA content (µg) and age of cod larvae: (a) starved larvae and larvae fed indirectly, through *Acartia* nauplii, on *Heterocapsa*, and *Rhodomonas* and (b) on *Skeletonema, Thalassiosira*, and a mixed 1:1 diet of *Skeletonema* and *Heterocapsa* (means and standard deviations of 5–10 individuals). Symbols as in Figure 1.

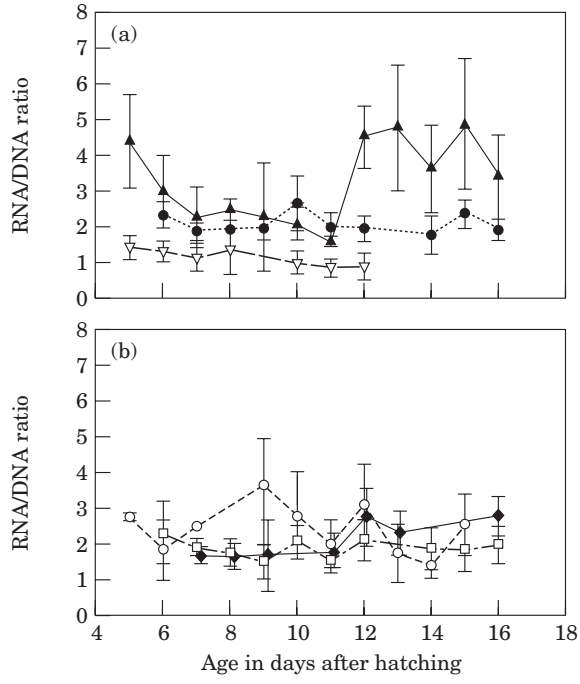

Figure 3. Relationship between RNA/DNA ratio and age of cod larvae: (a) starved larvae and larvae fed indirectly, through *Acartia* nauplii, on *Heterocapsa*, and *Rhodomonas* and (b) on *Skeletonema, Thalassiosira*, and a mixed 1:1 diet of *Skeletonema* and *Heterocapsa* (means and standard deviations of 5–10 individuals). Symbols as in Figure 1.

other groups had negative protein growth rates between −4.7% and −0.3% d^{-1}.

The fatty acid composition as a percentage of the total lipid content of the different algal species is given in Table 2. Larval growth rates were positively correlated with the relative contribution of DHA to total lipids [Figure 4(a)], with a negative correlation observed between larval growth rate and relative content of EPA [Figure 4(b)] and the ratio of ω3/ω6 fatty acids [Figure 4(c)].

Discussion

Prey type and composition are of great importance for growth and survival of larval fish. In particular, the nutritional quality of the food, as defined by the essential fatty acid composition, determines growth, behaviour, and development (Bell *et al.*, 1995; Sargent *et al.*, 1995). Because organisms belonging to higher trophic levels, including marine fish larvae, are incapable of the *de novo* synthesis of these highly unsaturated fatty acids, they depend on phytoplankton sources for normal

Table 2. Fatty acid composition as a percentage of total fatty acid of algae used in the experiments.

	Skeletonema (n=2)		*Thalassiosira* (n=7)		*Rhodonemas* (n=5)		*Heterocapsa* (n=9)	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
C14:0	50.8	66.8	79.6	74.4	28.7	8.8	447.9	277.4
C16:0	79.7	40.9	107.7	54.6	45.9	16.1	1 176.7	714.6
C18:0	3.5	0.1	5.8	5.7	9.5	11.9	300.0	237.6
Total saturates	134.1	25.7	192.3	132.2	84.1	33.6	1 924.5	1 227.3
C16:1ω7	192.3	60.9	157.7	106.7	16.6	11.2	99.3	40.7
C18:1ω9	2.1	0.1	14.5	26.4	13.2	7.9	78.0	44.0
C18:1ω7	17.5	3.8	10.3	7.6	24.5	7.3		
C20:1ω9	3.6	1.6	13.2	7.4				
C22:1ω9	1.7	0.2					883.2	1 633.5
Total monoenes	217.2	55.9	185.1	143.8	54.3	23.6	1 068.1	1 625.9
C18:2ω6	8.5	4.3	17.2	39.6	52.9	77.4	89.8	43.2
C20:4ω6	5.6	0.9			3.5	2.4		
Total nω6	14.0	3.4	18.1	39.8	55.0	79.6	89.8	43.2
C18:3ω3	60.3	32.8	56.3	37.2	100.2	72.7	429.9	286.0
C18:4ω3	23.7	15.3	29.2	16.2	11.5	13.6	344.0	181.0
C20:5ω3 (EPA)	237.1	136.8	138.3	64.8	65.4	16.3	28.8	10.9
C22:6ω3 (DHA)	35.9	13.3	32.9	18.8	33.6	3.2	1 013.0	431.9
Total nω3	368.9	214.9	262.6	121.6	211.8	96.2	1 802.9	892.8
Total FA	734.2	248.4	658.1	420.2	405.2	209.5	4 885.3	2 609.2
RATIO ω3/ω6	25.2	9.3	68.3	36.6	7.9	4.2	20.3	2.6
DHA/EPA	0.2	0.1	0.2	0.1	0.5	0.1	47.5	16.3
EPA % of total	30.9	8.2	22.5	3.8	17.8	5.0	0.3	0.3
DHA% of total	4.9	0.2	5.2	1.1	9.6	3.3	22.9	6.1

growth and development (Sargent *et al.*, 1995). The conservative transfer of dietary fatty acids between trophic levels has been identified previously (Lee *et al.*, 1971; St. John and Lund, 1996). Current estimates of EFA requirements of marine fish indicate that the ω3 EFA can only be met by 20:5ω3 (EPA) and 22:6ω3 (DHA) from phytoplankton sources (Sargent *et al.*, 1995).

To resolve variations in growth and condition of cod larvae in relation to feeding regime, we employed the RNA/DNA technique of Clemmesen (1993). The validity of the RNA/DNA ratio as an index of nutritional condition has been shown in numerous studies (Clemmesen, 1987, 1994; Ferron and Leggett, 1994; Westerman and Holt, 1994). The estimated growth rates and nutritional condition in our experiments were comparable to previous observations at similar feeding levels. For example, in a laboratory study, Buckley (1984) observed a Gpi of -1.3% per day for 2–11-day-old cod larvae fed on wild plankton (rotifers, copepod nauplii, copepodites) at a density of 0.2 organisms ml^{-1}. In a field study, Gpi of larval Baltic cod was estimated between -9% and 9% protein d^{-1} (Grønkjaer *et al.*, 1997). Thus, both studies yielded growth rates comparable to the range observed here (-4.7 to 4.0).

Several nutritional indices based on fatty acid content have been proposed for marine organisms. The ω3 to ω6 ratio has been suggested for fish (Tocher and Harvey, 1988) and crustaceans (Lytle *et al.*, 1990), as well as the content of EPA and DHA (Xu *et al.*, 1994). The importance of DHA and EPA for growth and reproductive success has been established for various marine species [e.g. oyster (*Crassostrea gigas*), Langdon and Waldock (1981), Thompson and Harrison (1992); turbot (*Scophthalamus maximus*), Scott and Middleton (1979); Chinese prawn (*Penaus chinensis*), Xu *et al.* (1994)]. Xu *et al.* (1994) warn that the ω3 to ω6 ratio may not be applicable to the Chinese prawn, and they state that further investigations are required. Their observation is supported by the weak though significant relationship ($p=0.006$) observed here. The relationship between growth and EPA and DHA is complex. We found a significant positive relationship ($p<0.001$) between growth of larval cod and DHA content of the algal source, and a significant negative relationship ($p<0.001$) between growth and EPA content. In contrast, growth in daphnids has been positively linked to EPA content

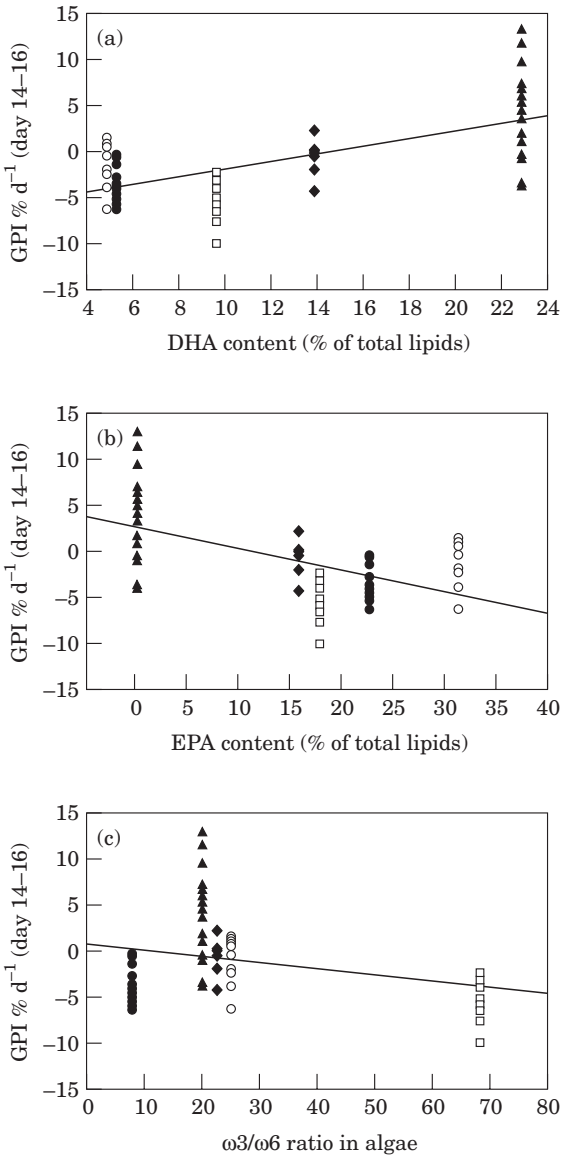

Figure 4. Mean Gpi of individual larval cod during day 14–16 in relation to (a) mean DHA content, (b) mean EPA content and (c) ω3/ω6 ratio of the algal food source for the five feeding regimes. Regression line (a: $y=0.40x-5.91$; $r^2=0.39$; $p<0.001$; b: $y=2.75x-0.24$; $r^2=0.30$; $p<0.001$; c: $y=-0.07x+0.75$; $r^2=0.11$; $p=0.006$) is fitted to the mean values. The values for the mixed diet are the means of the values for *Skeletonema* and *Heterocapsa*. Symbols as in Figure 1.

(Muller-Navarra *et al.*, 2000) and Langdon and Waldock (1981) observed that either appeared to fulfil the EFA requirements of *C. gigas* spat for growth. Further complicating the issue, Thompson and Harrison (1992) found an increase in growth rates in *C. gigas* with increase in dietary content of DHA. However, within the same study, an optimum level of EPA was observed beyond which a reduction in growth rate occurred. The lack of coherence in the results obtained so far suggests that the EFA composition of food in the marine environment is a complex issue potentially species-specific as well as varying during ontogeny, as suggested by Bell *et al.* (1995). Further research in this area is necessary.

Our main goal was to address the question of suitability of diatom production for higher trophic levels, and our results indicate that if toxic substances affect zooplankton production, the effects are not transferred further up the food chain to larval fish. A similar result was obtained for North Sea cod juveniles using diatom-specific lipid biomarkers (St. John and Lund, 1996). Our results indicate that variations in larval growth are related to EFA composition, in particular DHA, rather than to the transfer of toxic diatom substances, as concluded by Shaw *et al.* (1995, 1997) and inferred by others (Poulet *et al.*, 1995; Ianora *et al.*, 1995). The works by Shaw *et al.* (1995, 1997) and by Miralto *et al.* (1999) do not indicate if the toxins identified occur in the marine environment at concentrations that will influence growth and reproduction of higher trophic levels. Furthermore, research by these authors does not address the potential impacts of dietary deficiencies (Jónasdóttir and Kirboe, 1996).

The relationship between phytoplankton composition and growth of higher organisms is clearly a complex issue. Diatom production, particularly during the spring bloom, may constitute the dominant biomass available for herbivores. However, the suitability of these diatom cells for growth and reproduction varies in dependence with their dietary components and their potential toxicity. Our investigations suggest that, even though a single species may be deficient in a particular dietary component, a diet comprised of many different species and groups may negate the impact on higher trophic levels. However, the occurrence of an intense monospecific bloom may limit energy transfer to higher trophic levels because of dietary deficiencies or toxic constituents at some developmental stage during the bloom. The co-occurrence of fish larvae and such conditions may potentially result in windows of reduced survival success.

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