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Digestion of natural food by larval and post-larval turbot *Scophthalmus maximus*

D. V. P. Conway, P. R. G. Tranter, S. H. Coombs

Natural Environment Research Council, Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth, Devon PL1 3DH, United Kingdom

ABSTRACT: The digestion of natural, mainly crustacean zooplankton, by different age groups of turbot *Scophthalmus maximus* larvae was evaluated by comparisons of visual appearance, dry weight and carbon and nitrogen content of fresh food organisms with material recovered from faeces. Visually, the degree of digestion of food particles ranged from no discernible change of lamellibranch larvae, copepod eggs, intact copepod faecal pellets and some phytoplankton species, to varying degrees of removal of body constituents in copepods, cladocerans and decapod zoea. For crustaceans, the proportion of body constituents removed was related to the size and construction of their apparently indigestible exoskeleton. Upon defaecation larger organisms showed the greatest percentage loss in dry weight and carbon. A high percentage of nitrogen was extracted from all organisms. There was no consistent difference in digestive efficiency between different age groups of larvae.

INTRODUCTION

Starvation is suggested to be one of the primary factors influencing the high mortality experienced by the early larval stages of marine fish (Bailey & Houde 1989). Although considerable research effort has been directed towards understanding the relationship between survival of larvae and food availability, the digestibility of the various food organisms (i.e. how efficiently their constituents are removed during gut passage) has received little consideration.

Although they can consume a wide spectrum of food organisms (Turner 1984), the main food for larval fish is crustacean, especially the various developmental stages of copepods. Their diet generally reflects the species composition of the surrounding plankton (Checkley 1982, Young & Davis 1992). There can be great morphological and structural diversity and differences in chemical composition between the various food organisms, which must give equivalent variations in digestibility and nutritional potential. Furthermore, larval fish, with few reported exceptions (e.g. Rösch & Segner 1990), do not mechanically disrupt their prey during ingestion. Thus, while soft-bodied prey may be easily broken down enzymatically in the gut, organisms with apparently indigestible exoskeletons or shells, such as crustaceans and mollusc larvae, may be more difficult to digest efficiently and there may be a range of digestibility between prey types. Larval fish growth and survival could thus vary regionally and temporally due to differences in the digestibility of the most abundant available food.

Assessment of the ability of larval fish to digest particular organisms from conventional gut content investigations on preserved larvae is misleading, because as they are still within the digestive tract, digestion has not been completed. In the present study food organisms from freshly recovered faeces of different age groups of turbot *Scophthalmus maximus* larvae are examined and analysed to compare the efficiency of feeding on different items.

MATERIALS AND METHODS

Larval turbot, 17 and 34 d post-hatch were obtained from Golden Sea Produce Ltd, Hunterston, Scotland, in August 1992 and transported in insulated containers to Plymouth, England. The 34 d old turbot were undergoing metamorphosis but, for ease of description, all

ages are termed larvae. Experiments were subsequently carried out on 3 age ranges of larvae, 21 to 27 d (6.2 to 10.6 mm, ca 100 larvae), 37 to 46 d (14.4 to 20.5 mm, ca 60 larvae) and 53 to 67 d (21.6 to 39.2 mm, ca 30 larvae). While it would have been desirable to have also worked with a younger, potentially more vulnerable age range of larvae, there would have been practical difficulties in carrying out some of the procedures because of the small size of their food.

At the Plymouth Laboratory the larvae were maintained under continuous subdued lighting conditions, at temperatures of 18 to 19 °C, in small aquaria of 5 or 15 l, at a stocking density of 5 to 25 per aquarium, depending on size. The aquaria were not aerated but half the seawater (salinity approximately 34.0 psu) was replaced each day. Prior to transport to Plymouth the 17 d old larvae had been fed rotifers *Branchionus plicatilis* and *Artemia salina* nauplii, and the 34 d old larvae *A. salina* nauplii alone. These diets were replaced in the experiments with a mixture of wild zooplankton, collected regularly off Plymouth using a variety of plankton nets (50 to 200 µm). Some additional feeding experiments were carried out using the brackish water copepod *Eurytemora velox*. The range of organisms fed to the turbot larvae was, apart from *E. velox*, a similar mixture to that which they would encounter, and on which they have been observed feeding in the wild (Jones 1972, Last 1979). All experimental observations were made between 11:00 and 20:00 h GMT to reduce the influence of any diurnal changes in feeding intensity.

Feeding experiments were started at 07:00 h GMT when mixed plankton was introduced to each aquarium at a density of approximately 200 organisms l^{-1}. Plankton density was regularly maintained throughout the experiment and dead material removed. Collection of faecal material from the larvae was facilitated by turbot larvae producing faeces which are encased in a thin membrane which maintains their structural integrity. (Membranes were also observed on the faeces of *Gobius* sp. but not *Callionymus lyra* larvae, which had been collected in the plankton samples taken for food.) Over the first 4 h of each experiment, faeces were collected from the bottom of the aquaria using a wide-bore pipette and discarded. After 4 h, once the guts had been flushed out with more recently ingested food, faeces were collected at 5 min intervals. They were immediately opened in seawater under a microscope and a selection of intact individual organisms (n = 1717) removed for processing. A record was kept of the visual state of digestion of the organisms, as well as organism size (cephalothorax length for copepods, carapace length for decapod zoea, total length for cladocera and diameter for copepod eggs). They were then briefly dipped in distilled water to remove adherent salt. This procedure may lead to the loss of small amounts of organic material but is necessary in order to obtain accurate dry weights to which the other analyses are related. Organisms were then placed in solvent-cleaned (acetone and chloroform) preweighed (Cahn 25 Electrobalance) tin cups (5.3 × 3.2 mm). Depending on the weight of the organism, a variable number of specimens was placed in each cup to give a minimum of 20 µg sample weight. The open cups were then dried for 24 h at 60 °C, compacted, and stored in a desiccator. Subsequently the samples were weighed and then analysed for carbon and nitrogen with a Carlo Erba model NA 1500 Series 2 elemental analyser, using acetanylide as a calibration standard. Faecal membranes from 2 age groups of larvae were also collected and analysed.

In order to measure the dry weight and carbon and nitrogen content of undigested plankton, specimens (n = 947) of the same range of species as found in the faeces were selected from the same fresh plankton as supplied as food to the larvae. Processing of these samples was in the same way as for those extracted from the faeces.

During the experiments a restricted number of observations (n = 14) were made on the rate of passage of food particles through the intestinal tract. This was carried out by placing groups of 5 larvae in aquaria, feeding them until they were producing faeces and then introducing a different, easily recognisable marker food (e.g. *Eurytemora velox*). Feeding was then continued and faeces collected and examined at 10 min intervals until the first appearance of the marker.

As a measure of general larval condition and as a check on the functional integrity of the gut, samples of individual larvae (n = 24) were taken at intervals during the experiments and preserved in Baker's formol calcium fixative for subsequent histological examination. Following fixation, larvae were processed for methacrylate embedding, then serially sectioned at 2 µm in the sagittal plane, using Ralph glass knives, and stained in Lee's methylene blue/basic fuchsin, before mounting in Canada Balsam (McFadzen et al. 1991).

RESULTS

Feeding behaviour and rate of passage of food

Turbot larvae fed on a wide variety of prey, reflecting availability in the size range which they could ingest. From observations through the sides of the aquaria during the experiments it was noted that larger food particles were selected preferentially before smaller ones. This was especially the case for very motile and visible organisms such as decapod

zoea, although these were sometimes ejected from the mouth several times before being swallowed, probably a consequence of their spiny carapace and vigorous struggling.

The rate of passage of food through the guts of larvae was unpredictable and took between 1.2 and 6.3 h (Fig. 1). The main pulse of marker particles usually occured about 1 h after the first appearance. Food ingested concurrently did not necessarily pass through at the same rate; on 2 occasions *Eurytemora velox* eggs were observed in the faeces of 46 and 64 d old larvae, 50 min and 1 h respectively, before the adult female exoskeleton.

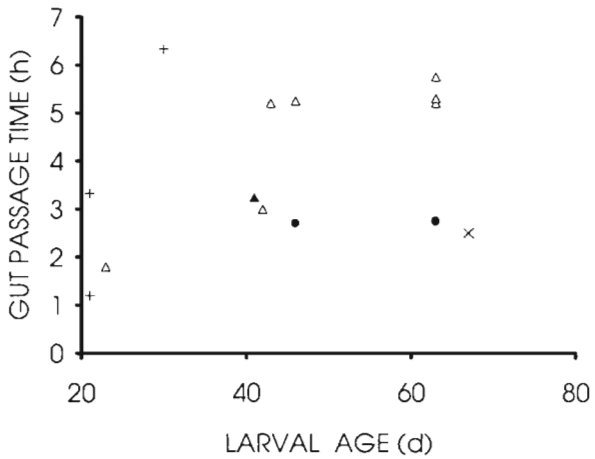

Fig. 1. *Scophthalmus maximus*. Rate of passage of a range of food items, through the intestinal tracts of larvae of different ages. Each point represents the fastest rate for a group of 5 larvae. (△) *Eurytemora velox*; (▲) *Anomalocera patersoni*; (●) *Temora longicornia*; (×) decapod zoea; (+) mixed plankton, including cirripede nauplii and the copepods *Pseudocalanus elongatus*, *Temora longicornis* and *Oithona* spp.

Histological examination of the gut

Histological examination of the digestive tract of turbot larvae from each of the 3 age groups showed that the fore-, mid- and hindgut regions were in good condition. Deep longitudinal folds (villi) were evident throughout the gut, particularly in the ventral region, which is indicative of normal healthy development (Cousin et al. 1986). In particular, the hindgut epithelium showed normal cellular integrity, with large supranuclear inclusion bodies present which are reported to be indicative of intracellular digestion of food particles engulfed by pinocytosis (O'Connell 1976). Other tissues assessed were the liver, pancreas, kidney, gills, trunk muscle, notochord and cartilage. All tissues were found to be healthy, in accordance with descriptions of Cousin et al. (1986).

Contents of the faeces

The membrane surrounding the faeces was usually tinted orange or brown. Faeces were usually well compacted, but a large proportion of crustacean exoskeletons were intact, although sometimes crushed and distorted. The largest copepods consumed were *Anomalocera patersoni*, *Labidocera wollastoni* and *Calanus helgolandicus* (Table 1) which were often broken into 2 or 3 pieces in the faeces, suffering greater disruption to the exoskeleton than smaller copepods. Of the smaller copepods, robust compact species such as *Centropages typicus*, *Temora longicornis*, *Corycaeus anglicus*, *Euterpina acutifrons* and *Oncaea* spp. were rarely crushed, while *Acartia clausi* and the cladoceran *Evadne nordmanni*, which are less robust, were usually crushed.

All nutrient digested from the food is not necessarily absorbed and an unknown and probably variable amount of dissolved nutrient, which cannot be quantified with the present experimental procedure, may be egested with the faeces and either immediately leach out into the aquarium or be released when the faecal membrane is removed. Results obtained are therefore a measure of the amount of material which the turbot larvae can digest from a particular organism and not what is actually absorbed.

Organisms in the faeces were usually well digested, with copepods reduced to transparent exoskeletons. There were, however, some exceptions. *Corycaeus anglicus* often appeared to be largely undigested, with orange liquid contents which were ejected if the copepod was punctured; *Temora longicornis* and *Centropages typicus*, which generally have very opaque bodies, sometimes had small amounts of undigested material remaining inside the exoskeleton. In *Pseudocalanus elongatus*, a species which often has large lipid reserves, lipid globules sometimes still remained in the exoskeleton, or occasionally globules were found free inside the faecal membrane when these copepods were present. In *Acartia clausi* especially, intact undigested faecal pellets liberated from the copepods gut were commonly observed free within the otherwise transparent exoskeleton. The subitaneous (non-diapause) eggs of *A. clausi*, *Eurytemora velox*, *Euterpina acutifrons*, *Oncaea* spp. and *C. anglicus* and the thick walled diapause eggs of *Evadne nordmanni* were commonly found apparently undigested in the faeces (Conway unpubl.).

Some non-crustacean food also appeared to resist digestion. Of the mollusc larvae, most lamellibranchs did not appear to be digested, only a few were noted with open shells and no contents. A few chitinous

Table 1. *Scophthalmus maximus*. Length range, mean dry wt of fresh food organisms and mean percentage loss in weight of the same range of food organisms after digestion by 3 age groups of larvae. Number of observations (n) are shown and also the standard deviations (SD) of the means where there was sufficient sample size. The developmental stage and sex (M = male and F = female) of copepods are noted

Organism	Length range (mm)	Mean fresh dry wt (µg)	SD	n	Mean % loss in weight after digestion					
					21-27 d	n	37-46 d	n	53-67 d	n
Copepoda										
Anomalocera patersoni 5M	1.8-1.9	129.4	-	2	-	-	87.6	2	-	-
A. patersoni 6F	1.8-2.8	253.9	27.9	4	86.8	1	84.5	6	-	-
A. patersoni 6M	2.2-2.4	185.3	12.0	5	-	-	85.6	7	82.7	1
Calanus helgolandicus 6F	1.8-2.3	107.5	7.9	6	-	-	85.1	2	85.1	2
C. helgolandicus 6M	2.0-2.2	128.0	-	1	84.4	1	79.7	1	-	-
Labidocera wollastoni 6F	1.8-2.4	132.2	-	2	-	-	89.6	3	81.1	1
L. wollastoni 6M	1.7-2.0	87.0	-	1	-	-	79.3	2	88.5	1
Centropages typicus 5F	0.8-0.9	20.4	-	2	-	-	87.7	1	60.8	1
C. typicus 6F	1.3-1.5	35.4	8.8	4	-	-	59.3	7	72.9	3
C. typicus 6M	1.1-1.3	35.8	2.9	4	-	-	72.1	2	69.0	4
Temora longicornis 5	0.7-0.8	8.6	-	1	-	-	40.7	3	44.2	2
T. longicornis 6F	0.8-0.9	22.1	0.8	4	-	-	65.2	4	69.7	19
T. longicornis 6M	0.7-0.9	14.5	-	2	-	-	-	-	60.0	21
Eurytemora velox 5F	0.6-1.0	15.5	1.4	3	80.6	5	-	-	-	-
E. velox 5M	0.6-0.8	12.0	-	1	81.7	2	-	-	-	-
E. velox 6F	0.9-1.1	16.9	1.8	11	78.7	4	-	-	79.3	2
E. velox 6F with eggs	1.0-1.1	19.3	1.5	9	-	-	-	-	-	-
E. velox 6M	0.7-1.0	14.8	1.4	9	77.0	3	-	-	70.9	2
E. velox eggs	0.09-0.11	0.3	-	2	-	-	-	-	-	-
Pseudocalanus elongatus 6F	0.7-0.08	9.0	0.3	3	-	-	75.6	1	-	-
Corycaeus anglicus 6F	0.5-0.7	8.1	0.1	3	-	-	64.2	5	54.3	4
Acartia clausi 6F	0.7-0.9	5.3	-	2	49.1	2	35.8	4	49.1	6
A. clausi 6M	0.7-0.9	8.1	1.6	3	-	-	67.9	1	72.8	2
Paracalanus parvus 5	0.6-0.7	5.3	-	1	73.6	1	-	-	-	-
P. parvus 6F	0.6-0.8	5.4	-	1	-	-	-	-	48.1	1
Euterpina acutifrons 6F	0.5-0.7	3.6	0.6	3	-	-	27.8	3	47.2	2
Cladocera										
Podon intermedius	0.7-0.9	9.7	0.7	3	-	-	80.4	3	56.7	2
Evadne nordmanni	0.4-0.5	3.1	0.7	3	45.2	3	48.4	3	77.4	1
Decapod zoea										
Necora puber	1.3-1.6	84.7	30.7	3	-	-	55.0	3	-	-
Pisidia longicornis	0.7-0.9	18.7	-	1	-	-	52.9	1	-	-
Faecal membrane										
37-46 d larvae	-	-	-	-	-	-	-	2	-	-
57-67 d larvae	-	-	-	-	-	-	-	-	-	15

chaetognath jaws were found, the only part of the organism apparently resistant to digestion. Occasionally the large diatom *Coscinodiscus concinnus* and the prasinophyte *Halosphaera* spp. occurred in the faeces, to all appearances undigested and still with green contents, although the contents were noticeably disrupted and the cells no longer viable.

The length of digested copepods was found to be slightly less than for undigested material. For example a group of *Eurytemora velox* females which were measured had a cephalothorax length range of 0.92 to 1.13 mm and a mean length of 1.01 ± 0.03 mm (n = 21) while the same stage after digestion had a cephalothorax length range of 0.81 to 1.01 mm and a mean length of 0.93 ± 0.04 mm (n = 45), a significant (from *t*-test analysis $p < 0.001$) reduction in length of 7.9%. The observed shrinkage may be due to partial collapse of the exoskeleton after loss of turgidity or to denaturation of the protein holding the exoskeleton together. The shrinkage, which is comparable to that found after fixation in formalin, is a consideration if one was trying to relate gut content measurements to fresh food measurements.

Dry weight analysis of fresh and digested food

The length range of fresh food organisms, their mean dry weight and their mean percentage loss in weight after digestion are given in Table 1. There were con-

siderable differences in mean fresh dry weight between male and female copepods of the same species and between species of the same cephalothorax length. Egg-bearing female copepods are not necessarily substantially heavier than those without eggs, as demonstrated by the similarity in mean dry weight of *Eurytemora velox* Stage 6 females with and without eggs. By weighing detached egg masses, individual egg weight was estimated to be 0.3 µg. The highest number of eggs counted in an egg mass was 29, so that the egg mass could represent up to to 57% of the mean body dry weight (16.9 µg).

The mean percentage loss in dry weight by food after digestion varied from 27.8 to 89.6% between different groups of food organisms. In general there was little difference in digestive weight loss of food between fish larvae of different ages. Only for the cladocerans was there any great variability, but with no consistent pattern. Weight loss was highest (>80%) in the largest, heaviest copepods such as *Calanus helgolandicus*, *Anomalocera patersoni* and *Labidocera wollastoni*. Among the smaller copepods (*Pseudocalanus elongatus*, *Acartia clausi*, *Corycaeus anglicus*, *Paracalanus parvus* and *Euterpina acutifrons*), weight loss after digestion was more variable at between 27.8 and 75.6%. Both species of decapod zoea had low weight loss (52.9 and 55.0%) even though *Necora puber* had a fresh weight comparable to the larger copepods which showed a high weight loss. Within the different stages of the same copepod species percentage weight loss was generally similar.

Carbon and nitrogen analysis of fresh and digested food

The mean percentage carbon and nitrogen of the dry weight of fresh food organisms and their mean percentage loss in carbon and nitrogen following digestion, for the same group of specimens as in Table 1, are given in Tables 2 & 3. The range of percentage carbon for fresh copepods varied from 28.2 to 52.4% and while there was no clear relationship with size, lower values found tended to be among the smaller copepods such as *Euterpina acutifrons*. On passage through the guts of turbot larvae, copepods lost 55.7 to 97.7% of their carbon, in the majority of cases decreasing by over 80%. Larger copepods tended to lose a greater percentage. A high proportion of carbon (80.2 to 89.5%) was also removed from cladocerans and the decapod zoea *Necora puber*, although in the other zoea, *Pisida longicornis*, it was reduced by a lesser amount (64.8%). There was no clear difference in digestive ability between larvae of different ages although the older turbot larvae tended to extract a greater percentage of carbon.

The proportion of the fresh dry weight of copepods represented by nitrogen ranged from 6.4 to 11.5% (Table 3). In general the smaller copepods had the lower mean percentages. A very high proportion of nitrogen was extracted during digestion (78.7 to 100%) and in approximately half the analyses it was not detectable in the faeces. There were no clear patterns between different organisms, stages of copepods or ages of larvae. The mean C:N ratio for fresh organisms (Table 3) varied for most species over a narrow range from 3.6 to 4.5, though the high carbon content of *Eurytemora velox* led to C:N values of up to 5.3.

When the individual determinations of percentage carbon and nitrogen of fresh and digested food are plotted against dry weight for 4 large (Fig. 2a, b) and 4 small copepod species (Fig. 3a, b) the effects of digestion are highlighted. In fresh copepods the carbon and nitrogen values fall within a restricted range. The greatest variability, especially in percentage carbon, was among copepods weighing <20 µg. The greater variability in carbon and nitrogen composition observed among smaller, lighter organisms is not a problem of analytical sensitivity because the smaller items were bulked together to give similar sample weight to larger organisms, but reflects real differences in chemical composition and differences related to structural diversity. In digested food (Figs. 2b & 3b) the spread of values is much greater than in fresh food. Greater variability was again in food remains weighing <20 µg where a large proportion of specimens had low or no detectable nitrogen.

Faecal packaging does not appear to occur in all fish larvae and whatever advantage is gained has to be balanced against energy lost in the process. The amount of carbon and nitrogen lost as a percentage of the dry weight of the faecal membrane (Tables 2 & 3) was very low (14.4 to 14.7% and 0 to 1.1% respectively), so the membrane has no more nutritional potential than the faecal constituents.

DISCUSSION

Larval fish have been shown, both in experimental studies and in the field, to lose condition when food is scarce (Werner & Blaxter 1980, Canino et al. 1991). In the present study turbot larvae were fed zooplankton at approximately 200 l^{-1} which is a higher concentration than found under most natural conditions (Canino et al. 1991, Coombs et al. 1992). The feeding concentration was chosen so that larvae were not constrained by lack of food and with the potential for an element of selectivity. Higher concentrations were not offered

Table 2. *Scophthalmus maximus*. Mean weight of carbon and mean percentage carbon of the dry weight of fresh food organisms and mean percentage loss in carbon of the same range of food organisms after digestion by 3 age groups of larvae. The number of observations are as for Table 1. Standard deviations (SD) of the means are shown where there was sufficient sample size. The developmental stage and sex (M = male and F = female) of copepods are noted

Organism	Mean fresh weight of carbon (µg)	SD	Mean % carbon of fresh dry wt	SD	Mean % loss in carbon after digestion		
					21–27 d	37–46 d	53–67 d
Copepoda							
Anomalocera patersoni 5M	49.3	–	38.0	–	–	92.9	–
A. patersoni 6F	102.7	12.2	39.8	0.7	91.8	93.2	–
A. patersoni 6M	76.2	6.6	40.7	1.0	–	91.6	89.3
Calanus helgolandicus 6F	42.4	5.6	38.7	2.5	–	94.8	88.7
C. helgolandicus 6M	49.3	–	38.5	–	91.5	87.8	–
Labidocera wollastoni 6F	52.4	–	42.1	–	–	89.5	91.2
L. wollastoni 6M	35.5	–	40.8	–	–	90.0	90.2
Centropages typicus 5F	10.6	–	34.2	–	–	92.4	91.2
C. typicus 6F	14.4	4.3	40.3	3.4	–	81.9	84.7
C. typicus 6M	13.2	0.6	36.4	3.4	–	71.7	84.3
Temora longicornis 5	3.2	–	37.4	–	–	68.3	79.3
T. longicornis 6F	9.3	0.6	42.1	3.0	–	80.6	82.7
T. longicornis 6M	5.4	–	37.7	–	–	–	72.3
Eurytemora velox 5F	7.8	0.3	50.3	3.4	93.3	–	–
E. velox 5M	6.3	–	52.4	–	97.7	–	–
E. velox 6F	7.2	0.9	43.1	5.4	90.9	–	94.6
E. velox 6F with eggs	9.2	0.8	47.6	1.2	–	–	–
E. velox 6M	7.0	0.8	47.7	4.5	94.5	–	93.9
E. velox eggs	0.1	–	35.1	–	–	–	–
Pseudocalanus elongatus 6F	3.7	0.2	42.1	3.0	–	94.5	–
Corycaeus anglicus 6F	2.8	0.2	34.0	2.2	–	72.0	80.5
Acartia clausi 6F	2.4	–	44.7	–	74.1	64.9	84.2
A. clausi 6M	2.4	0.1	31.9	8.8	–	87.5	85.7
Paraclanus parvus 5	1.6	–	30.6	–	83.0	–	–
P. parvus 6F	1.7	–	32.0	–	–	–	75.9
Euterpina acutifrons 6F	1.0	0.2	28.2	3.0	–	55.7	74.9
Cladocera							
Podon intermedius	3.3	0.3	34.1	3.2	–	85.6	–
Evadne nordmanni	1.2	0.2	39.6	1.9	83.1	80.2	87.4
Decapod zoea							
Necora puber	26.9	8.0	33.2	3.1	–	89.5	–
Pisidia longicornis	4.7	–	25.4	–	–	64.8	–
Faecal membrane							
37–46 d larvae	–	–	14.4	–	–	–	–
57–67 d larvae	–	–	14.7	4.6	–	–	–

since an excess of food is suggested to lead to supression of digestion (Werner & Blaxter 1980). The larvae preferentially fed on the largest suitable organism available, which has also been noted in other work on turbot (Meeren 1991) and, in common with most larval fish feeding studies, food organisms appeared to be ingested without any obvious mechanical disruption.

One measure of condition of fish larvae is the state of the cells lining the intestine, as estimated from histological criteria (e.g. Oozeki et al. 1989). During starvation the cell height of the gut wall reduces, enzyme production declines and thus digestive capability diminishes (Pedersen et al. 1990) and food may be egested undigested. In the present study, histological sections of the gut and other organs of larval turbot confirmed that the larvae were in good condition. It may thus be concluded that there was no undue feeding stress on the larvae and that adequate nutrition was available.

The extent to which food organisms were digested depended on their size and structure. The larger copepods which have a strong exoskeleton were often broken into 2 or 3 pieces. Because of their bulk they are probably more easily crushed by peristaltic action as they pass through the gut, although some of the disruption could also have taken place as they passed through the anal sphincter. Among the smaller species of copepods, the degree to which they were crushed in the faeces depended on the thickness of the exoskeleton and the compactness of their body. Species which

Table 3. *Scophthalmus maximus*. Mean weight of nitrogen and mean percentage nitrogen of the dry weight and C:N ratio of fresh food organisms, and mean percentage loss in nitrogen of the same range of food organisms after digestion by 3 age groups of larvae. The number of observations are as for Table 1. Standard deviations (SD) of the means are shown where there was sufficient sample size. The developmental stage and sex (M = male and F = female) of copepods are noted

Organism	Mean fresh weight of nitrogen (µg)	SD	Mean % nitrogen of fresh dry wt	SD	C:N ratio	Mean % loss in nitrogen after digestion 21–27 d	37–46 d	53–67 d
Copepoda								
Anomalocera patersoni 5M	13.7	–	10.6	–	3.6	–	100.0	–
A. patersoni 6F	27.2	3.3	10.5	0.2	3.8	94.5	95.1	–
A. patersoni 6M	21.0	2.0	11.2	0.5	3.6	–	95.2	93.2
Calanus helgolandicus 6F	11.5	1.5	10.5	0.7	3.7	–	100.0	97.6
C. helgolandicus 6M	12.5	–	9.8	–	3.9	100.0	92.3	–
Labidocera wollastoni 6F	14.3	–	11.5	–	3.7	–	100.0	93.0
L. wollastoni 6M	10.0	–	11.4	–	3.6	–	100.0	100.0
Centropages typicus 5F	2.4	–	7.8	–	4.4	–	100.0	100.0
C. typicus 6F	3.5	1.0	9.7	0.5	4.1	–	97.1	94.2
C. typicus 6M	3.4	0.2	9.5	1.2	3.8	–	81.8	95.1
Temora longicornis 5	0.8	–	9.8	–	3.8	–	97.0	94.9
T. longicornis 6F	2.4	0.1	10.7	0.4	3.9	–	87.6	86.7
T. longicornis 6M	1.4	–	9.7	–	3.9	–	–	80.2
Eurytemora velox 5F	1.7	0.1	10.9	0.9	4.6	100.0	–	–
E. velox 5M	1.2	–	9.9	–	5.3	100.0	–	–
E. velox 6F	1.8	0.2	10.8	1.4	4.0	100.0	–	99.0
E. velox 6F with eggs	2.1	0.2	11.2	0.3	4.2	–	–	–
E. velox 6M	1.5	0.1	10.2	0.8	4.7	100.0	–	98.9
E. velox eggs	0.027	–	8.3	–	4.2	–	–	–
Pseudocalanus elongatus 6F	0.9	0.1	10.8	1.0	3.9	–	100.0	–
Corycaeus anglicus 6F	0.6	0.1	8.1	0.6	4.2	–	78.7	95.1
Acartia clausi 6F	0.6	–	10.9	–	4.1	82.2	94.5	98.1
A. clausi 6M	0.6	0.1	8.0	1.8	3.9	–	97.2	96.9
Paracalanus parvus 5	0.4	–	7.4	–	4.1	100.0	–	–
P. parvus 6F	0.4	–	8.4	–	3.8	–	–	100.0
Euterpina acutifrons 6F	0.2	0.1	6.4	0.9	4.5	–	92.1	100.0
Cladocera								
Podon intermedius	0.7	0.1	7.6	0.7	4.5	–	100.0	–
Evadne nordmanni	0.3	0.1	10.7	1.2	3.7	100.0	100.0	100.0
Decapod zoea								
Necora puber	6.7	1.7	8.5	1.6	4.0	–	94.3	–
Pisidia longicornis	1.1	–	5.9	–	4.3	–	100.0	–
Faecal membrane								
37–46 d larvae	–	–	0.0	–	–	–	–	–
57–67 d larvae	–	–	1.1	1.2	–	–	–	–

occasionally still had small amounts of obviously undigested contents were generally those which were resistant to crushing and thus from which it may be more difficult to extract their contents. While *Corycaeus anglicus* seemed particularly resistant to digestion, this was not completely reflected in the dry weight and chemical analysis of digested specimens.

From an examination of the faeces it is clear that particular components in the diet are poorly digested by turbot larvae. Chitinous crustacean exoskeletons at least superficially appear to be unchanged by the digestion process. Few studies have analysed for chitinolytic enzyme activity in fish larvae, although chitinase has been reported in the eggs and larvae of red sea bream *Pagrus major* (Kono et al. 1987) and from the late yolk sac stage and beyond in trout *Salmo gairdneri* (Lindsay 1985). Even in adult fish which feed on crustaceans, chitin does not appear to be fully utilized (Seiderer et al. 1987). Lindsay (1984) concluded that, in adult fish, the primary function of chitinase may be for the initial chemical disruption of the exoskeleton of prey. This may also be the function in larval fish, although some nutritional gain cannot be discounted.

Lipid absorption has been demonstrated in many larval fish species (Rösch & Segner 1990, Deplano et al. 1991) and turbot larvae have been shown to possess the enzyme lipase (Cousins et al. 1987). However, in this study, turbot larvae had apparently not extracted all the storage lipid from *Pseudocalanus elongatus*.

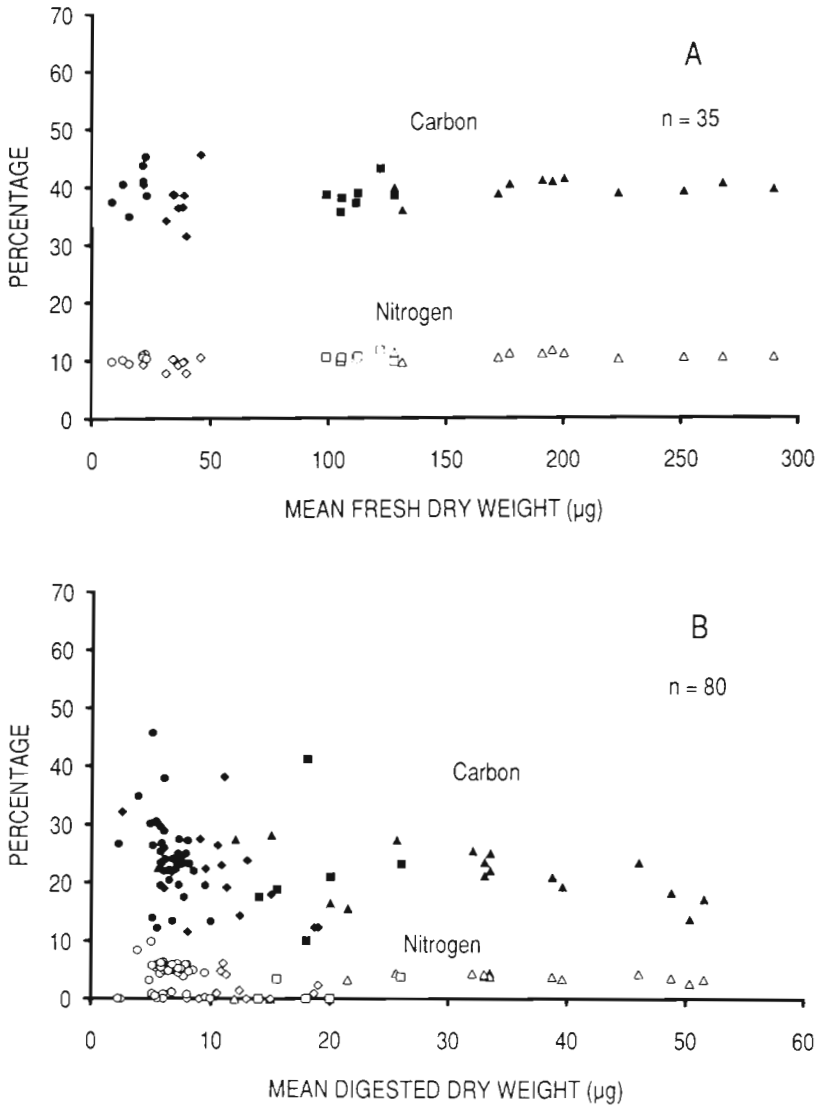

Fig. 2. *Scophthalmus maximus*. Percentage carbon and nitrogen of the individual mean dry weights of 4 of the larger copepods taken as food: (A) fresh and (B) after digestion. No. of observations (n) for each analysis are given. (▲, △) *Anomalocera patersoni*; (■, □) *Calanus helgolandicus*; (◆, ◇) *Centropages typicus*; (●, ○) *Temora longicornis*

Checkley (1982) and Pedersen (1984) also noted poor digestion of copepod storage lipid in larval herring *Clupea harengus*. Wax ester, the main storage lipid of copepods, is more difficult to digest than other lipid classes (Patton et al. 1975) so that when large quantities appear in the gut, larvae may not have the enzyme capacity to cope with it.

Certain complete organisms were almost invariably found apparently unaltered by the digestion process. These included lamellibranch larvae whose resistance to digestion by larval fish is well documented and has been noted by Bhattacharyya (1957), Spectrova et al. (1974), Checkley (1982), Kane (1984), Tilseth & Ellertsen (1984), Nakata (1988) and Viñas (1991). Lamellibranch larvae are numerous in the diet of fish larvae in particular areas (Checkley 1982, Brewer & Kleppel 1986, Nakata 1988) and there is evidence that they were actively selected by Black Sea turbot *Scophthalmus maeoticus* (Spectrova et al. 1974).

The observed resistance of phytoplankton to digestion has also been noted by Nakata (1988) for diatoms, by Checkley (1982) for prasinophytes and by Kane (1984), Nakata (1988) and Viñas (1991) for dinoflagellates. Digestion of phytoplankton in many fish larvae may be limited to species with less robust cell walls. Phytoplankton has been observed in the guts of many larvae at the early stages of development although it is commonly reported as an amorphous mass (Last 1979, Kane 1984). Copepod faecal pellets are also a source of phytoplankton material in larval fish guts (Ellertsen et al. 1980). In the present study and in that by Bhattacharyya (1957) there was no visual evidence of copepod faecal pellets being digested. The chemical composition of the pellet membrane is still controversial, but chitin is considered to be a major component (Bochdansky & Herndl 1992). Since the membrane is closed at both ends it forms an unbroken barrier against enzyme penetration. However, when the pellets have been free in the sea for some time and been exposed to bacterial and mechanical disruption (Lampitt et al. 1990), they may be more digestible.

Because of the variability of copepod morphology there were considerable dry weight differences between species and between sexes. It is clear that body dimension measurements would have to be more comprehensive than cephalothorax length alone to be able to relate them accurately to weight. Reproductive status could also affect dry weight. However the mean dry weight of Stage 6 females of *Eurytemora velox* was similar whether or not they were carrying eggs, even though the egg mass can represent up to 57% of the mean body dry weight. This is understandable because some of the females were carrying few eggs,

while some without eggs had many late development eggs already in the oviducts ready to be laid, and so were effectively carrying them internally.

There was little clear difference in digestion efficiency with increasing larval age, which is surprising since digestive enzyme complement has been demonstrated to change with age in turbot larvae (Cousin et al. 1987), suggesting that over the age range of larvae examined, the enzyme systems are similarly developed.

After digestion the heavier organisms tended to have the highest percentage weight loss (>80%). It appears that while the exoskeleton of crustaceans becomes thicker and heavier with increasing size, the increasing ratio of body volume to surface area outweighs this, so that the weight proportion of digestible internal contents increases. There are exceptions to this as in the decapod zoea; *Necora puber* has a high dry weight but lost only 55.0% on digestion, which is low compared with a copepod of the same weight, probably due to differences in exoskeleton bulk. Among the smaller crustaceans the harpacticoid copepod species *Euterpina acutifrons* had a low weight loss (27.8 to 47.2%) which may be due to their small size, particular cylindrical body shape, and thus high exoskeleton weight relative to contents.

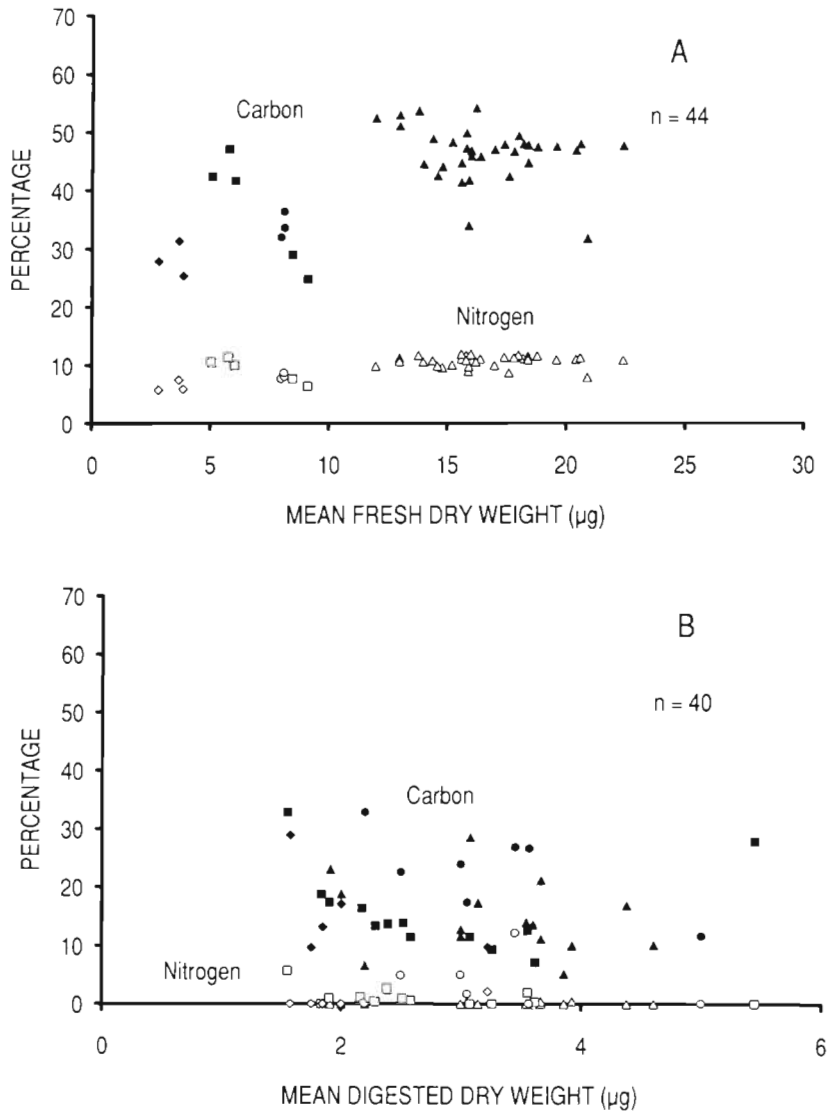

Fig. 3. *Scophthalmus maximus*. Percentage carbon and nitrogen of the individual mean dry weights of 4 of the smaller copepods taken as food: (A) fresh and (B) after digestion. No. of observations (n) for each analysis are given. (▲, △) *Eurytemora velox*; (■, □) *Acartia clausi*; (◆, ◇) *Euterpina acutifrons*; (●, ○) *Corycaeus anglicus*

Pedersen & Hjelmeland (1988) estimated that 82 to 95% of carbon was extracted from mixtures of *Acartia tonsa* nauplii and early copepodite developmental stages during digestion by larval herring. These results are similar to the present study where reduction in carbon content on digestion reflected the reduction in dry weight, being greater in the larger copepods (>85%) and less, and more variable, in small copepods and decapod zoea. Since turbot larvae were observed to preferentially select the larger food items, this represents an efficient feeding strategy. Nitrogen compounds appear to be more readily extractable components than carbon; in half the analyses nitrogen was reduced to the level where it was not detectable. Nitrogen is associated with the soft parts of the prey while the chitinous exoskeleton is high in structural carbon. Chitin contains low levels of nitrogen and this will contribute to the value measured in digested food. Zero nitrogen values suggest either that there is partial digestion of chitin or that values are below detection limits. No clear pattern of reduction in nitrogen was seen between different organisms.

Irrespective of the degree of digestibility of different food items, the actual amount of food absorbed is the important factor in the nutrition of larval fish. Rate of food passage through the gut, which can be modified by many factors (Karjalainen et al. 1991), may be an

important determinant of the efficiency of food absorption. Experimental results have indicated that passage rate can be altered by changing the food concentration, such that *Artemia salina* nauplii fed at high densities were still alive after passing through the gut of larval herring *Clupea harengus*, whereas they were digested at lower densities (Werner & Blaxter 1980). Similar observations were made on Black Sea turbot *Scophthalmus maeoticus* (Spectorova et al. 1974). Boehlert & Yoklavich (1984) found for larval Pacific herring (*C. harengus pallasi*) that, while evacuation rate increased with increasing food concentration, carbon assimilation from individual food particles decreased; however, overall, the greater food throughput gave a higher energy gain. In the present series of experiments, where food concentration was relatively high, the greatest variable possibly being the species composition of the plankton supplied as food, gut passage time was variable and unpredictable. However, food organisms were invariably well digested.

The rate of passage of different sizes of food organisms through the larval fish digestive tract is poorly understood. In larval herring *Clupea harengus*, which have straight guts, Pedersen (1984) observed that copepods did not change their position relative to one another. However, peristaltic action has been observed to alter the relative position of food in larval cod *Gadus morhua* of 7 d post-hatch, which at this age have simple straight tubular guts (Tilseth & Ellertsen 1984). In the present study, while passage rates were unpredictable, particles of different sizes, ingested at the same time, such as *Eurytemora velox* and their eggs, were observed to have different rates in the looped gut of turbot larvae. Karjalainen et al. (1991) observed that when feeding of larval vendace *Coregonus albula* was suspended, copepodites remained in the guts longer than nauplii, also suggesting slower passage rates of larger organisms. Possibly, differential retention of food by larval fish is more evident at even later stages of gut development.

The results from this study demonstrate, for larval turbot, a considerable range in digestibility of natural food both within and between prey species. However, data were collected over several weeks and factors such as changes in individual condition of larvae, differences in nutritional quality within and between food species, the species mix of food made available and the possibility that some organisms spend longer passing through the gut than others, could all interact to produce the variability observed in the results. These are all factors the larvae would be subjected to in nature and therefore the results may be representative of natural conditions.

Fish larvae feed on a variety of organisms and stages and while particular food organisms may be of lower nutritional potential than others, present results suggest that it is unlikely under conditions of high food concentration and broad species diversity that their nutritional requirements would not be largely satisfied. It is possible that if food density was at best marginal to their requirements and this was coupled with a large proportion being poorly digestible, they could suffer loss in condition, causing death through starvation or an increased vulnerability to predation. Digestibility of food may thus be a contributory factor in larval fish survival.

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