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PHYSIOLOGICAL AND BIOCHEMICAL ENERGETICS OF LARVAE OF *TEREDO NAVALIS* L. AND *BANKIA GOULDI* (Bartsch) (BIVALVIA : TEREDINIDAE)[1]

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Abstract: The biochemical composition of larvae of *Teredo navalis* L. and *Bankia gouldi* (Bartsch) (Bivalvia : Teredinidae) was examined throughout larval development at 23 °C and 30–32‰ salinity in the presence of the phytoplankton food *Isochrysis* aff. *galbana* (clone T-ISO), during a delay of metamorphosis in the presence of food but absence of a wood substratum and during periods of enforced starvation. Newly released *Teredo navalis* larvae had a mean length (L) and height (H) of 89.3 and 76.1 μm respectively. The first appearance of pediveliger larvae at 212.1 μm L and 230.0 μm H occurred 27 days after release. Larval dry weight increased from 0.29 μg to 1.96 μg during this period. Newly formed straight hinge larvae of *Bankia gouldi* had dimensions of 62.8 μm L and 49.8 μm H. Metamorphically competent *B. gouldi* larvae had dimensions of 230.0 μm L and 282.9 μm H and were first observed 20 days after fertilization. Larval dry weight increased from 0.06 μg to 2.20 μg during this period. During enforced delay of metamorphosis the ash-free dry weight of *Teredo navalis* larvae decreased whereas the ash free dry weight of *Bankia gouldi* larvae increased. During the early period of shelled larval development both species showed similar decreases in lipid, protein and carbohydrate levels ($\mu g \cdot mg\ dry\ weight^{-1}$); however, this was reflected in a decrease in biochemical content ($\mu g \cdot larva^{-1}$) only in *Teredo navalis*. During enforced starvation the major proportion of both the weight and caloric losses were due to protein. Lipid also contributes significantly to these losses whereas the contribution of carbohydrate was small. Larval oxygen consumption rates were determined directly by manometry and indirectly by estimates of decrease in caloric content during periods of enforced starvation. Direct and indirect determinations for *T. navalis* are described by the relationships $R = 1.16\ W^{1.05}$ and $R = 0.98\ W^{1.24}$ respectively where R is the respiration rate in nl $O_2 \cdot larva^{-1} \cdot h^{-1}$ and W is dry weight inclusive of shell in μg. Direct and indirect determinations for *Bankia gouldi* are described by the relationships $R = 1.37\ W^{1.25}$ and $R = 1.81\ W^{1.25}$ respectively. When data for both assay procedures are combined for each species the relationships $R = 1.10\ W^{1.07}$ and $R = 1.44\ W^{1.18}$ are obtained for *Teredo navalis* and *Bankia gouldi* respectively.

Key words: *Teredo navalis*; *Bankia gouldi*; larvae; physiology; biochemistry

INTRODUCTION

Historical interest in the marine wood boring molluscs of the family Teredinidae and the subfamily Martesiinae has been stimulated by their destruction of man-made wooden structures in the marine environment. Early contributions include the works of Sellius (1733), Forbes & Hanley (1853), Tryon (1862), Jeffries (1865), Sigerfoos (1908) and a host of more recent work summarized in the excellent works of Clapp & Kenk

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(1963) and Turner (1966). These and more recent studies (for example, Culliney, 1975; Boyle & Turner, 1976) have shown that larvae of the Teredinidae and Martesiinae are generally typical of marine bivalve larvae in their morphology and planktivorous feeding. A notable exception exists in the genus *Lyrodus* which broods larvae to a pediveliger stage (sensu Carriker, 1961) which is competent to metamorphose shortly after release. During settlement on and initial penetration of a wood substratum considerable morphological change occurs resulting in the unusual vermiform shape that characterizes adult Teredinidae (see Turner, 1966 for an excellent discussion of the comparative anatomy of the adult Teredinids).

Measurements of physiological activity in both the larval and adult teredinids have been few. Only one study, that of Gallager *et al.* (1981) on the brooding species *Lyrodus pedicellatus*, has attempted to formulate a complete energy budget for the adult animal. The relative contributions of wood, phytoplankton and possibly dissolved organic material (D.O.M.) to energy balance in post metamorphic teredinids remains a subject of active discussion (Mann, 1984). This paucity of data provided the stimulus for a study to compare and contrast energy balance in the larval and post metamorphic (adult) forms of the teredinids and eventually to provide data on the relative roles of wood, phytoplankton and D.O.M. in the nutrition of adult animals. The study focussed initially on the oviparous *Bankia gouldi* (Bartsch) and, by contrast, on *Teredo navalis* L. which broods larvae to the straight hinge veliger stage prior to release, in order to provide a comparison of species exhibiting different reproductive habits. In this report we describe the first component of the aforementioned study, namely an examination of growth, oxygen consumption, and changes in biochemical composition in the larvae of *T. navalis* and *Bankia gouldi* during periods of abundant food and enforced starvation.

Materials and Methods

Adult *Teredo navalis* were collected from fir test panels (5 × 10 × 20 cm) suspended in ≈10 m of water at the dockside in Great Harbor, Woods Hole, MA. Natural settlement of *T. navalis* at this site occurs from May through October (see Grave, 1928; Coe, 1933; Grave & Smith, 1936). Adult *Bankia gouldi* were collected from similar test panels suspended from the dock of the Duke University Marine Laboratory, Pivers Island, Beaufort, N. C. Test panels from this site were transported by air to Woods Hole in cool, insulated containers. Panels containing test species were maintained separately in sea-water aquaria (2.5 × 1 × 0.25 m) supplied with flowing, unfiltered sea water of 30–32‰ salinity which was pumped directly from Vineyard Sound, MA. Sea-water temperature was maintained at 20 ± 2 °C by heating through the fall, winter and spring months. During the summer ambient temperature water was supplied, this reaching a maximum temperature of 23 °C in August.

Teredo navalis released larvae throughout the year under laboratory conditions. Larvae were collected on a 53 μm "Nitex" nylon mesh sieve held below the outflow of

the aquaria holding the spawning adults and transferred to larval culture vessels within 1 h of release.

Bankia gouldi were stimulated to spawn by thermal shock. The copulatory behavior noted in Turner (1966) was observed regularly prior to spawning. On initiation of spawning fertilized eggs were gently collected in a large bulb pipette, transferred to a prewashed container, subsequently rinsed on a 20 μm "Nitex" nylon mesh, and transferred to the larval culture container.

Depending upon the numbers of larvae obtained and the experimental objectives, larvae were cultured in glass containers of either 3 l or 12 l capacity, or in linear polyethylene (Nalgene) cylindrical tanks of 50 l or 200 l capacity. All water for the larval cultures was filtered to 0.45 μm by serial passage through 10- and 1-μm orlon wound cartridge filters (Carborundum Company) and a pleated membrane 0.45-μm filter (Filtrite Corporation) and equilibrated to culture temperature prior to use. All cultures were effected at ambient salinity of 30–32‰, and 23 °C. Larval densities were maintained in the range 1–10 larvae $\cdot$ ml^{-1}, decreasing from highest density at the first veliger stage to lowest density at late umbo or pediveliger stage. Water in the larval cultures was changed every 2 or 3 days depending upon the species (see Tables I and II). At this time larvae were collected from the culture by siphoning through a suitable size "Nitex" nylon mesh sieve, washed into a glass finger bowl with 0.45-μm filtered sea water, examined under a dissecting microscope, a subsample removed and preserved in 10% buffered formalin for subsequent length (anterior-posterior axis) and height (dorso-ventral axis) determination, and the remainder returned to the refilled culture vessel. Length and height measurements were made on a Leitz compound microscope fitted with an ocular micrometer. Further subsamples for biochemical analysis or for the starvation experiments were also removed, as required, at the water changes. At each water change culture vessels were washed thoroughly with soap and hot fresh water before refilling. After returning the larvae to the culture vessel sufficient phytoplankton food (*Isochrysis* aff. *galbana*, clone T-ISO) was added to give a resulting food concentration of 5×10^4 cells $\cdot$ ml^{-1}. This food level has been shown to stimulate maximum grazing rates in *Teredo navalis* larvae (Gallager & Mann, 1980). Gentle aeration was provided to all cultures as recommended by Helm & Spencer (1972).

Where starvation periods formed an integral part of the experimental design, they were effected as above but without the addition of food.

The flagellate *Isochrysis* aff. *galbana* (clone T-ISO) was used exclusively for larval culture. The taxonomic designation of this species remains tentative. Ewart & Epifanio (1981) summarize information on the original isolation and work to that date. No further comments have appeared (Dr. R. R. L. Guillard, pers. comm.). In previous unpublished experiments this species supported growth of both *Teredo navalis* and *Bankia gouldi* larvae which was comparable to or better than that obtained on mixtures of *Pavlova* (*Monochrysis*) *lutheri* (Droop Green) (formerly *Monochrysis lutherii* Droop), *Tetraselmis suecica* (Kylin) Butch, *Thalassiosira pseudonana* Hassel et Heimdal (Husdedt) (clone 3H) and *Isochrysis galbana* Parke. Furthermore, the upper temperature tolerance of

I. galbana (clone T-ISO) is higher than the aforementioned species thus facilitating culture maintenance. Axenic stocks of *I. galbana* (clone T-ISO) were obtained from the collection of Dr. R. R. L. Guillard, maintained in the F/2 media of Guillard & Ryther (1962) and subcultured at regular intervals. Axenic cultures of 50 ml were used to inocculate 3-l Fernbach flasks or 12-l glass carboys containing 0.22 μm filtered, autoclaved sea water enriched with f/2. Phytoplankton was cultured at 23 °C under continuous illumination from a bank of 4 × 40 W "Spiralux" fluorescent bulbs. The 12-l cultures were gently aerated with filtered air. Both 3- and 12-l cultures were harvested for feeding during the exponential phase of growth. All cell counts were made by hemacytometer on a Leitz compound microscope.

All determinations of biochemical composition for each species were made on larvae originating from one culture. At regular intervals larvae were obtained at a water change and either prepared directly for analysis or starved for a period of 3–7 days to allow comparison of actively feeding larvae and larvae that were required to utilize stored reserve material. Preparation for analysis consisted of quickly rinsing the larvae in isoosmotic ammonium formate (3% w/v), washing the larvae into a glass vial, freeze drying and subsequent storage in sealed vials in a desiccator at 0 °C. Individual weight determinations were made on a Perkin Elmer microbalance. The larvae were subsequently ground to a powder in an agate mortar and pestle.

Ash content was determined as that remaining after ignition to constant weight at 450 °C. Ash free dry weight is the difference between dry weight and ash weight.

Carbon and nitrogen contents were determined using a Perkin Elmer Carbon-Hydrogen-Nitrogen Analyzer.

The procedures for lipid, carbohydrate and protein assays are illustrated in Appendix 1. Assays were made on an initial homogenate of $\approx$ 10 mg of whole freeze dried larvae in 1000 μl of distilled, deionized water. Homogenization was effected using a Branson sonifier, this method giving consistently better replication than glass homogenizers. For the lipid assay replicate aliquots of the homogenate were extracted in 1 : 2 v/v chloroform : methanol (Bligh & Dyer, 1959) followed by a second extraction in 2 : 1 v/v chloroform : methanol (Folch *et al.*, 1957). Purification of the lipid containing chloroform layer was effected using 0.7% w/v NaCl solution (Marsh & Weinstein, 1966). Lipid was quantified by both the sulphuric acid charring technique of Marsh & Weinstein (1966) with calibration versus tripalmitin, and gravimetrically. Both techniques gave comparable results, but the latter was preferred in that it eliminated possible charring of the lipid during the drying step of the Marsh & Weinstein (1966) procedure. Such charring resulted in a suspension of floccular material on addition of sulphuric acid and gave unsatisfactory assays. The data reported here are from gravimetric assays.

Carbohydrate and protein assay began with extraction of the initial water homogenate with trichloracetic acid to give a final concentration of 5% w/v after mixing. After standing overnight at 4 °C and centrifuging (10 min at $\approx$ 1000 *g*) the supernatant was removed for carbohydrate assay. This extraction follows Holland & Gabbott (1971), Holland & Hannant (1973) and Mann (1979a) in assuming that particulate protein and

nucleic acids are precipitated while free amino acids, metabolic intermediates and all sugars (including polysaccharides) remain in solution. Thus is in conflict with Roberts *et al.* (1963) who state that only simple sugars are extracted by cold T.C.A. and that extraction of complex polysaccharides requires hot 10% w/v T.C.A. The data of Mann (1979 a,b) suggest, however, that the major storage polysaccharide, glycogen, is extracted by cold 5% w/v T.C.A. in homogenized bivalve tissue. The cold extraction technique employed here was therefore deemed adequate. The carbohydrate content of the supernatant was assayed by the phenol-sulphuric acid method of Raymont *et al.* (1963) using glucose as a standard. The protein content of the precipitate was assayed by the Folin-phenol reaction of Lowry *et al.* (1951) using bovine albumen as a standard. Protein assays were only completed on *Teredo navalis* larvae. These data are referred to as protein content to distinguish them from protein estimates, calculated from $\%N \times 6.25$, which were made for *Bankia gouldi* (see Tables I and II).

Caloric content was estimated both directly using a Parr microbomb calorimeter, calibrated with sodium benzoate and correcting for calcium carbonate as suggested by Payne (1966), and indirectly from the biochemical data using the caloric equivalent of 9.45, 5.65 and 4.1 cal/mg for lipid, protein and carbohydrate respectively (Crisp, 1971).

Indirect estimates of oxygen consumption rate are made from the sum of daily caloric losses of each biochemical component using the following conversion factors as reported by Elliot & Davison (1975): carbohydrate, $3.53\ \text{cal} \cdot \text{mg}\ O_2^{-1}$; lipid, $3.28\ \text{cal} \cdot \text{mg}\ O_2^{-1}$; protein, $3.20\ \text{cal} \cdot \text{mg}\ O_2$ consumed^{-1}. The value for protein assumes degradation of protein to ammonia, an assumption supported by demonstration of ammonia excretion by shipworm larvae (Mann & Gallager, unpubl. data). Volumetric estimates were obtained from gravimetric values by multiplication by 1.4.

All direct measurements of larval respiration were made using a modified version of the differential microrespirometer of Grunbaum *et al.* (1955). The modification consisted of increasing the flask volume from ≈ 2 to 5 ml and changing the shape from an exaggerated flat bottom to spherical. When a 2-ml working volume of 0.45-μm filtered sea water was used in assays the modified flask allowed active swimming of bivalve larvae in contrast to the original design which, when filled with 1 ml of water, caused larvae to be trapped by surface tension at the periphery of the flask.

At regular intervals throughout development a subsample of larvae was removed from the culture, retained on an appropriate sized "Nitex" nylon mesh, rinsed with 0.45-μm filtered sea water of the same salinity (30–32‰) and temperature (23 °C) as used in larval culture, and transferred to the respirometer flask. Assays used between 100 and 1000 larvae, the number decreasing with increasing size of larvae, in 2 ml of 0.45 μm filtered sea water in the absence of food organisms. Throughout the stabilization and assay period respirometers were immersed in a temperature controlled water bath at 23 °C. After an initial stabilization period of 30 min the manometers were sealed and readings commenced. Readings were taken every 30 min for 4 h. Computation of oxygen consumption rate was made using the initial and final manometric readings and was restricted to those assays which exhibited consistent rates of change of manometric

readings throughout the 4-h period. Assays exhibiting erratic rates of change were discarded. On completion of an assay larvae were removed, fixed in 10% buffered formalin, counted, and measured for length (anterior-posterior axis) and height (dorso-ventral axis) on a Leitz compound microscope fitted with an ocular micrometer. Estimates of individual larval dry weight were made from weight and length data reported in Tables I and II. Appropriate blank assays containing 2 ml of 0.45-μm filtered sea water but without larvae were included in all experimental trials.

RESULTS

Data for growth and biochemical composition of *Teredo navalis* larvae throughout development and during enforced starvation are summarized in Table I. Newly released

TABLE I

Growth and biochemical composition of *Teredo navalis* larvae throughout development at 23 °C and 30 – 32‰ salinity on a diet of *Isochrysis* aff. *galbana* (clone T-ISO), and during periods of enforced starvation at 23 °C and 30–32‰ salinity: *, insufficient sample to complete analysis; n.d., not determined; BD, below detection.

Age (days) from release	Days fed	Days starved	L μm	H μm	Dry wt (μg·larva^{-1})	% ash	Ash free dry wt (μg·larva^{-1})	Composition (μg·mg dry wt^{-1}) carbo-hydrate	lipid	protein (Lowry)	C	N
0	0	0	89.3	76.1	0.29	84.0	0.047	16.2	35.5	84.9	223	21.5
3	3	0	101.2	86.5	0.30	*	*	7.4	18.9	57.0	*	*
6	6	0	105.2	95.0	0.36	86.0	0.051	*	18.9	*	*	*
9	9	0	121.6	122.9	0.49	79.0	0.103	8.4	18.3	76.2	*	*
12	12	0	141.2	146.0	0.62	72.0	0.174	7.6	15.0	71.7	151	13.4
15	15	0	158.2	164.5	0.77	74.3	0.199	11.4	18.9	143.8	177	19.6
18	18	0	180.5	196.2	1.10	61.8	0.422	12.9	19.9	133.8	*	*
21	21	0	199.4	217.9	1.41	50.8	0.709	14.2	22.3	139.4	206	26.7
24	24	0	208.9	226.4	1.59	72.8	0.432	12.2	19.0	141.2	*	*
27	27	0	212.1	230.0	1.96	67.9	0.629	10.3	23.6	97.2	*	*
30	30	0	216.0	238.4	2.12	61.8	0.809	8.3	36.1	92.0	*	*
40	40	0	218.0	240.2	2.04	75.0	0.510	7.5	25.0	93.3	243	34.0
3	0	3	n.d.	n.d	0.20	86.8	0.026	5.1	12.0	69.0	165	13.9
6	3	3	n.d.	n.d	0.29	*	*	5.6	12.3	39.0	*	*
9	6	3	n.d.	n.d	0.31	79.2	0.066	*	12.3	*	*	*
12	9	3	n.d.	n.d	0.34	86.4	0.047	5.9	16.1	67.8	*	*
15	12	3	n.d.	n.d	0.48	71.7	0.136	4.0	10.1	55.7	149	13.1
18	15	3	n.d.	n.d	0.62	*	*	9.1	17.2	110.6	185	21.6
21	18	3	n.d.	n.d	0.99	78.8	0.209	9.6	8.0	112.4	*	*
24	21	3	n.d.	n.d	1.18	78.9	0.249	8.4	18.5	95.4	188	24.8
27	24	3	n.d.	n.d	1.55	74.6	0.394	11.0	17.0	99.3	*	*
7	0	7	95.9	84.3	0.18	88.8	0.019	3.15	*	42.4	*	*
10	0	10	100.1	87.8	0.15	92.2	0.009	*	6.3	16.5	*	*
13	0	13	100.2	86.9	0.14	94.9	0.007	*	*	*	*	*
18	0	18	100.1	87.0	0.14	94.7	0.007	*	6.2	*	*	*

larvae had a mean length (L) and height (H) of 89.3 and 76.1 μm, respectively. Equidimensional larvae of ≈ 122 μm length and height were observed 9 days after release. The first appearance of pediveliger larvae, at 212.1 μm L, and 230.0 μm H, occurred 27 days after release. During a subsequent enforced delay of metamorphosis for 13 days in the presence of food but absence of wood substratum only very small increments in length and height were recorded. Larval dry weight increased from 0.29 μg at release to 1.96 μg for the first pediveliger. Ash-free dry weight during the same period increased from 0.047 $\mu g \cdot larva^{-1}$ to 0.629 $\mu g \cdot larva^{-1}$. During enforced delay of metamorphosis, mean dry weight changed very little, but ash-free dry weight decreased to 0.510 $\mu g \cdot larva^{-1}$.

Newly released *T. navalis* larvae showed a decrease in lipid, carbohydrate and protein content during the first 3 days of development. After this time gradual increases in all

Caloric content (cal · mg dry wt^{-1})		Biochemical content (ng · larva^{-1})					Caloric content (cal · larva^{-1}) ($\times 10^{-4}$)	
calculated	measured	carbo-hydrate	lipid	protein (Lowry)	C	N	calculated	measured
0.88	1.95	4.73	10.37	24.7	65.1	6.27	2.6	5.6
0.53	*	2.22	5.67	17.1	*	*	1.6	*
*	*	*	6.82	*	*	*	*	*
0.64	*	4.12	8.22	37.3	*	*	3.1	*
0.58	*	4.72	9.32	44.5	93.8	8.32	3.7	*
1.04	1.02	8.78	14.55	110.7	136.3	15.09	8.2	9.1
1.00	1.00	14.24	21.97	147.7	*	*	11.3	11.0
1.06	0.72	20.02	31.44	196.6	290.5	37.65	15.3	10.1
1.03	0.86	19.36	30.15	224.1	*	*	16.8	13.6
0.81	*	20.19	46.26	190.5	*	*	16.3	*
0.89	*	17.60	76.53	195.0	*	*	19.4	*
0.79	*	15.31	51.03	190.4	495.9	69.39	16.6	*
0.52	0.28	1.02	2.40	13.8	33.0	2.78	1.1	0.6
0.34	*	1.62	3.56	11.3	*	*	1.1	*
*	0.11	*	3.87	*	*	*	*	0.3
0.56	0.57	2.02	5.52	23.3	*	*	2.0	2.0
0.43	*	1.92	4.85	26.7	71.5	6.29	2.1	*
0.82	1.46	5.64	10.66	68.6	114.7	13.39	5.3	7.1
0.75	0.85	9.49	7.91	111.2	*	*	7.7	8.4
0.75	0.80	9.91	21.83	112.6	221.8	29.3	9.1	9.4
0.77	*	17.05	26.35	153.9	*	*	12.3	*
*	*	0.55	*	7.4	*	*	*	*
*	*	*	0.91	2.4	*	*	*	*
*	0.09	*	*	*	*	*	*	*
*	0.04	*	0.84	BD	*	*	*	*

components were observed with protein content consistently exceeding lipid content and both exceeding carbohydrate content. During enforced starvation of *T. navalis* larvae for 3 days consistent losses were observed in dry weight and ash-free dry weight and in protein, lipid and carbohydrate content.

Data for growth and biochemical composition of *Bankia gouldi* larvae throughout larval development (0–40 days) and for subsamples starved for 3–7 days are given in Table II. Newly formed straight hinge larvae have mean dimensions of 62.8 μm *L*, and 49.8 μm *H*. At 9 days old the developing larvae were approximately equidimensional (71.4 μm *L* × 71.3 μm *H*). The first pediveliger stage to appear had mean dimensions of 160.9 μm *L* × 169.3 μm *H* and was observed at 19 days. Animals competent to metamorphose exhibit mean dimensions of 230.0 μm *L* × 282.9 μm *H* and were first observed on Day 28.

During larval development an increase in dry weight from 0.06 μg per larva at 2 days old to 2.20 μg per larva at 28 days was observed. Although only marginal increases in both length (2.0 μm) and height (3.2 μm) were recorded during a subsequent 12 days growth in the absence of a substratum, dry weight continued to increase to 2.86 μg. Ash-free dry weight increased from 0.028 $\mu g \cdot larva^{-1}$ at first shelled veliger to 0.887 $\mu g \cdot larva^{-1}$ at competent pediveliger. Unlike *Teredo navalis* larvae, the larvae of *Bankia gouldi* continued to increase in ash-free dry weight to 1.181 $\mu g \cdot larva^{-1}$ during enforced delay of metamorphosis.

TABLE II

Growth and biochemical composition of *Bankia gouldi* larvae throughout development at 23 °C and 30–32‰ salinity on a diet of *Isochrysis* aff. *galbana* (clone T-ISO), and during periods of enforced starvation at 23 °C and 30–32‰ salinity: *, insufficient sample to complete analysis.

Age (days)	Days fed	Days starved	L (μm)	H (μm)	Dry wt ($\mu g \cdot larva^{-1}$)	% ash	Ash free dry wt ($\mu g \cdot larva^{-1}$)	carbo-hydrate	Composition ($\mu g \cdot mg$ dry wt^{-1}) lipid	protein (N × 6.25)	C	N
2	2	0	62.8	49.8	0.06	52.3	0.028	8.4	49.0	167.5	210	26.8
6	6	0	68.1	57.4	0.17	63.0	0.063	4.4	46.0	113.7	181	18.2
9	9	0	71.4	70.3	0.19	53.7	0.088	4.8	32.0	126.8	180	20.3
13	13	0	112.5	115.1	0.52	53.4	0.242	4.8	44.0	133.7	183	21.4
19	19	0	160.9	169.3	0.71	58.4	0.295	4.7	31.2	124.3	180	19.9
28	28	0	230.0	282.9	2.20	59.7	0.887	9.3	71.0	246.8	255	39.5
40	40	0	232.0	286.1	2.86	58.7	1.181	13.4	75.0	235.6	264	37.7
7	2	5	65.3	52.3	0.04	87.6	0.005	4.3	36.0	30.0	140	4.8
9	6	3	69.6	58.9	0.15	67.0	0.049	1.4	19.0	87.5	163	14.0
12	9	3	72.9	71.8	0.17	61.1	0.066	2.1	25.0	99.3	163	15.9
17	13	4	114.0	117.0	0.21	67.2	0.068	4.0	22.0	88.7	165	14.2
23	19	4	162.9	171.3	0.54	64.0	0.194	3.5	14.0	84.4	159	13.5
31	28	3	231.5	284.4	2.00	50.8	0.984	2.5	49.0	174.3	215	27.9
47	40	7	235.0	289.6	2.54	59.7	1.024	9.4	*	223.7	247	35.8

Bankia gouldi showed a similar decrease to *Teredo navalis* in relative lipid, protein and carbohydrate composition ($\mu g \cdot$ mg dry wt^{-1}) during the early days of shelled larval development but unlike *T. navalis* the biochemical content increased during this period (compare Tables I and II). Throughout larval development estimated protein (N × 6.25) content consistently exceeded lipid content, and both exceeded carbohydrate content. Changes in length and height of *Bankia gouldi* larvae during enforced starvation were minimal; however, consistent decreases were observed in dry weight and ash-free dry weight and in protein, lipid and carbohydrate content.

During starvation a decrease in the content of a particular biochemical component is indicative of the use of that component as a respiratory substrate. In order to examine this process further in *Teredo navalis* and *Bankia gouldi* larvae, a comparison of the loss of the major components during starvation on both a weight and calculated caloric content basis was made. These data are summarized in Table III. A consistent trend is evident in both species with protein forming the major proportion of both the weight and caloric losses. Lipid also contributed a significant proportion of these losses. Of particular interest is the importance of lipid to newly released *Teredo navalis* larvae suggesting the importance of maternally derived lipid in the egg to early development. The contribution of carbohydrate is small. Where estimates of caloric loss are available from both direct calorimetry and component analysis a general agreement between the two data sets was seen.

The relationships of oxygen consumption rate, R(nl $O_2 \cdot$ larva$^{-1} \cdot h^{-1}$) versus dry weight $W(\mu g)$ per larva for *T. navalis* and *Bankia gouldi* are illustrated in Figs. 1 and

Caloric level (cal · mg dry wt^{-1})		Biochemical content (ng · larva^{-1})					Caloric content (cal · larva^{-1}) (× 10^{-4})	
calculated	measured	carbo-hydrate	lipid	protein (N × 6.25)	C	N	calculated	measured
1.40	1.34	0.49	2.84	9.7	12.1	1.55	0.8	0.8
1.07	*	0.75	7.82	19.3	30.7	3.09	1.9	*
1.01	0.81	0.91	6.08	24.1	34.2	3.86	2.0	1.5
1.16	0.55	2.51	22.88	69.5	95.1	11.12	6.2	2.9
0.99	0.74	3.34	22.15	88.2	127.8	14.13	9.5	5.3
2.05	1.78	20.37	156.20	542.9	561.0	86.91	46.5	39.2
2.05	2.29	38.24	214.50	673.8	755.0	107.82	66.3	65.5
0.52	0.29	0.17	1.44	1.2	5.6	0.19	0.2	0.1
0.66	*	0.22	2.85	13.1	24.4	2.10	1.0	*
0.65	0.67	0.36	4.25	16.9	27.7	2.70	1.4	1.1
0.71	0.17	0.83	4.62	18.6	34.6	2.98	1.5	0.4
0.61	0.38	1.86	7.56	45.5	85.9	3.93	3.4	2.1
1.42	1.02	5.04	98.00	348.6	430.0	55.80	29.2	20.4
*	2.10	23.83	*	568.2	627.4	90.93	*	53.3

TABLE III

Biochemical and energetic losses by *Teredo navalis* and *Bankia gouldi* larvae during starvation for 3–7 days (ng · larva^{-1} · day^{-1}): *, insufficient sample to complete analysis; n.d., not determined.

Teredo navalis

Age of larvae (days)	Dry wt (ng)	Ash free dry wt (ng)	Carbohydrate (ng)	Carbohydrate (cal × 10^{-6})	Lipid (ng)	Lipid (cal × 10^{-6})	Protein (ng)	Protein (cal × 10^{-6})	C (ng)	N (ng)	Caloric loss (cal × 10^{-6}) calculated	Caloric loss (cal × 10^{-6}) measured
0	30.6	7.0	0.41	1.69	2.66	25.1	3.63	20.4	10.7	1.16	47.2	n.d.
3	3.7	n.d.	0.20	0.82	0.70	6.6	1.93	10.9	n.d.	n.d.	18.3	n.d.
6	15.3	n.d.	n.d.	n.d.	0.98	9.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
9	49.0	18.7	0.69	2.85	0.90	8.5	4.66	26.3	n.d.	n.d.	37.7	n.d.
12	47.0	12.7	0.93	3.82	1.49	14.0	5.93	33.5	7.4	0.67	51.3	n.d.
15	50.0	n.d.	1.05	4.28	1.29	12.2	14.03	79.3	7.2	0.56	95.8	n.d.
18	38.3	71.0	1.58	6.49	4.68	44.2	12.17	68.7	n.d.	n.d.	119.4	n.d.
21	76.6	153.3	3.37	13.82	3.20	30.2	28.00	158.2	22.9	2.78	202.2	n.d.
24	12.3	12.7	0.77	3.16	1.27	11.9	23.40	132.2	n.d.	n.d.	147.3	n.d.

Bankia gouldi

Age of larvae (days)	Dry wt (ng)	Ash free dry wt (ng)	Carbohydrate (ng)	Carbohydrate (cal × 10^{-6})	Lipid (ng)	Lipid (cal × 10^{-6})	N × 6.25 (μg)	N × 6.25 (cal × 10^{-6})	C (μg)	N (μg)	Caloric loss (cal × 10^{-6}) calculated	Caloric loss (cal × 10^{-6}) measured
2	3.6	n.d.	0.06	0.26	0.28	2.6	1.70	9.6	1.3	0.27	12.5	13.1
6	6.6	4.6	0.18	0.73	1.66	15.7	2.06	11.2	2.1	0.33	27.6	n.d.
9	6.6	7.3	0.19	0.76	0.61	5.8	2.40	13.1	2.7	0.39	19.6	13.4
13	77.5	58.0	0.42	1.73	4.57	43.2	12.70	69.2	15.1	2.03	114.4	62.5
19	42.5	25.2	0.37	1.51	3.64	34.4	10.68	58.2	10.5	2.55	94.1	79.9
28	66.6	n.d.	5.11	20.95	19.40	183.3	64.77	353.0	43.6	10.37	557.3	625.3
40	45.7	22.4	2.06	8.44	n.d.	n.d.	15.08	82.2	18.2	2.41	n.d.	173.6

2 respectively. The linear regressions fitted to the data obtained manometrically and denoted thus (○) are described by the following relationships:

$$T.\ navalis\ R = 1.16\ W^{1.05},\ n = 18,$$
$$B.\ gouldi\ R = 1.37\ W^{1.25},\ n = 28,\ r = 0.767,\ P < 0.01.$$

Eight indirect estimates of oxygen consumption for *Teredo navalis* larvae in the dry weight range 0.29–1.59 μg were made and are denoted thus (△) on Fig. 1. Five such estimates were also made for *Bankia gouldi* larvae in the dry weight range 0.17–2.20 μg and are denoted thus (△) in Fig. 2. The linear regressions fitted to those data are described by the relationship:

$$T.\ navalis\ R = 0.98\ W^{1.24},\ n = 8,\ r = 0.919,\ P < 0.01$$
$$B.\ gouldi\ R = 1.81\ W^{1.25},\ n = 5,\ r = 0.980,\ P < 0.01.$$

Figs. 1 and 2 illustrate that the data sets for both assay procedures are comparable within a species. The consistency of the calculated exponent value is particularly notable. When the data for each species are combined the relationships can be described as follows:

$$T.\ navalis\ R = 1.10\ W^{1.07},\ n = 26,\ r = 0.822,\ P < 0.01$$
$$B.\ gouldi\ R = 1.44\ W^{1.18},\ n = 33,\ r = 0.847,\ P < 0.01.$$

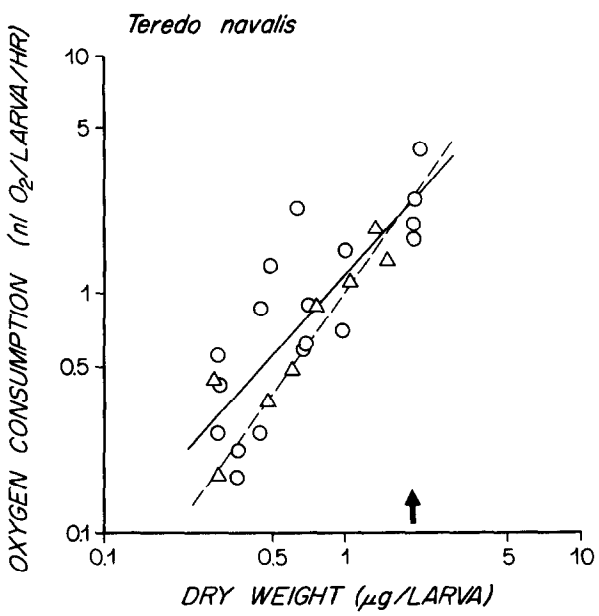

Fig. 1. Oxygen consumption rate (nl $O_2 \cdot larva^{-1} \cdot h^{-1}$) in relation to dry weight (μg) inclusive of shell in *Teredo navalis* larvae: points denoted (○) are from microrespirometer assays and are described by the solid regression line; points denoted (△) are indirect estimates from caloric loss during starvation and are described by the broken regression line (see text for details); arrow on the weight axis indicates size at which 50% of the larvae have a pediveliger foot.

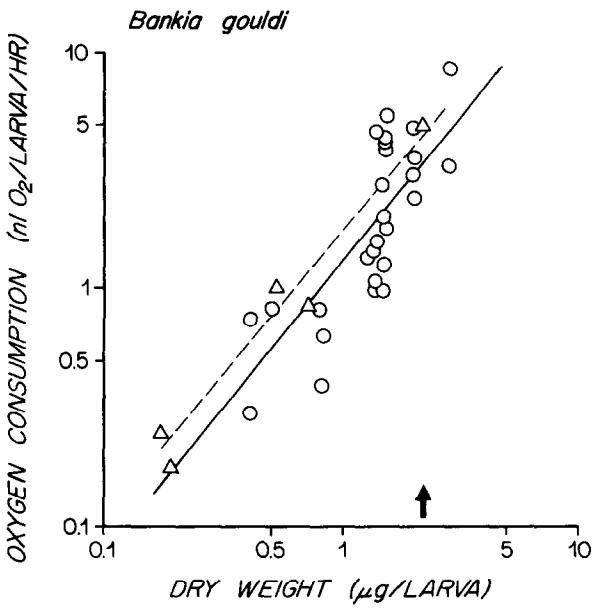

Fig. 2. Oxygen consumption rate ($nlO_2 \cdot larva^{-1} \cdot h^{-1}$) in relation to dry weight (μg) inclusive of shell in *Bankia gouldi* larvae: points denoted (○) are from microrespirometer assays and are described by the solid regression line; points denoted (△) are indirect estimates from caloric loss during starvation and are described by the broken regression line (see text for details); arrow on the weight axis indicates size at which 50% of the larvae have a pediveliger foot.

Discussion

Larval development and growth rates of *Teredo navalis* and *Bankia gouldi* have been described previously by Culliney (1975). Good agreement exists between the data of Culliney and this study for the size of the first shelled larvae stage and pediveliger larva. Culliney cultured both species at 25 °C and 30‰ on a diet of *Isochrysis galbana*. Under these conditions *Bankia gouldi* developed to pediveligers in 25 days, comparable to the present study; however, *Teredo navalis* developed to pediveligers in only 15 days, considerably faster than in the present study.

Holland & Spencer (1973) examined biochemical changes in fed and starved larvae of *Ostrea edulis* during larval development, metamorphosis and early spat growth. In healthy, actively growing *O. edulis* larval shell weight (estimated by Holland & Spencer to be the difference between total and ash free dry weight, here referred to as ash weight) decreased from 83% of the total weight in newly released larvae of 179 μm *L* to 73.5% at metamorphosis (296 μm *L*). These values are comparable to those recorded for *Teredo navalis* in this study but higher than those for *Bankia gouldi*. Values for the total dry weight per larva for *Ostrea edulis*, *Teredo navalis* and *Bankia gouldi* are comparable; however, some differences are evident in the composition data. *Ostrea edulis* larvae

generally had lower protein levels ($\mu g \cdot mg$ dry wt^{-1}) than *Bankia gouldi* larvae but higher levels than *Teredo navalis* larvae. This may be in part due to the different analytical techniques used for each species (Holland & Spencer used Kjeldahl nitrogen × 6.25 to estimate protein which is different from the method used in the present study). *Ostrea edulis* and *Bankia gouldi* larvae had comparable lipid levels ($\mu g \cdot mg$ dry wt^{-1}), as assayed by very similar techniques, but both exceeded *Teredo navalis* larval lipid values. The level of free reducing sugars (FRS) in *Ostrea edulis* larvae, assayed in a cold 5% w/v T.C.A. extract by alkaline ferricyanide reduction, were comparable to the present carbohydrate values for *Teredo navalis* larvae; however, further hot hydrolysis (1 N HCl, 95 °C for 2 h) of this extract from *Ostrea edulis* hydrolyzed some polysaccharide to produce total carbohydrate values (FRS plus polysaccharide) in excess of the values recorded for *Teredo navalis* larvae.

In short term starvation experiments Holland & Spencer (1973) showed that neutral lipid was the major energy reserve of larvae and young spat of *Ostrea edulis*. In a subsequent review Holland (1978) suggested that, due to its abundance and ease of mobilization, lipid is probably the major energy reserve in many marine invertebrate larvae. Previously Gallager & Mann (1981) noted that *Teredo navalis* larvae utilize lipid reserves under starvation stress; however, they did not effect simultaneous assays of protein content. Recently Mann & Gallager (1984) examined the changes in biochemical composition of the larvae of the oviparous pholad *Martesia cuneiformis* during 3-day starvation periods initiated when the larvae were 15 days (162.6 μm L × 160.8 μm H, 0.60 μg dry weight) and 35 days (260.4 μm L × 269.4 μm H, 1.72 μg dry weight) old. The losses of carbohydrate, lipid and protein (estimated as $\%N \times 6.25$) during the starvation period, when expressed as calories per larva, were 21.7×10^{-6}, 288×10^{-6} and 396.7×10^{-6} cal respectively for 15-day old larvae and 145.9×10^{-6}, 639.1×10^{-6} and 914.8×10^{-6} respectively for 35-day-old larvae. In *M. cuneiformis* larvae it was protein, not lipid, that formed the predominant energy reserve. The data of Table III similarly show the predominant role of protein rather than lipid as an energy reserve during larval development of both *Teredo navalis* and *Bankia gouldi*. The importance of parentally derived lipid, that is egg lipid, in early development has been noted by Helm *et al.* (1973) for *Ostrea edulis*. The high lipid level ($\mu g \cdot mg$ dry wt^{-1}) in both newly released *Teredo navalis* larvae and first shelled veligers of *Bankia gouldi* suggest a similar role for egg lipid in these teredinids.

Zeuthen (1947) suggests that the exponent value, b, relating metabolic rate functions to weight should, in small marine planktonic organisms, approximate to 1. This would be indicative of a direct relationship between metabolic rate and weight rather than surface area: volume ratio. The oxygen consumption values obtained manometrically and for pooled data in this study support this suggestion. Furthermore, there is good agreement between the absolute rates of oxygen consumption in *Teredo navalis* and *Bankia gouldi* larvae recorded here and rates for comparably sized larvae of other marine bivalves in the same temperature range irrespective of the method used to measure respiration (Table IV). The general agreement between direct and indirect estimates of

TABLE IV

A comparison of respiration rates in bivalve larvae as measured by different methods: A, Cartesian diver; B, differential manometric microrespirometer; C, oxygen electrode; D, estimated from caloric loss during starvation; length measurements are anterior-posterior axis; dry weight is inclusive of shell unless otherwise stated in the footnote; a and b are the constants in the relationship $R = aW^b$; n.s., information not stated.

Species	Length (μm)	Dry wt (μg)	nl · larva^{-1} · h^{-1}	°C	a	b	Method	Reference
Mytilus edulis	235–250	n.d.	0.5 – 1.6	n.s.	[f]	[f]	A	Zeuthen, 1947
Mytilus edulis	151–238	0.1 –0.5[a]	0.39– 1.66	15	3.10	0.90	A	Riisgard *et al.*, 1981
Crassostrea virginica	60–200	n.d.	0.2 –20.0	26	n.d.	n.d.	B	MacInnes & Thurberg, 1973
Martesia cuneiformis	69–250	0.2 –1.6	0.09–10.1	22	2.22	2.42	B	Mann & Gallager, 1984
Teredo navalis	89–216	0.29–2.12	0.16– 4.0	23	1.16	1.05	B	This study
Bankia gouldi	98–232	0.41–2.8	0.78– 9.1	23	1.37	1.25	B	This study
Ostrea edulis	219	2.44[b]	5.0[d]	22	[f]	[f]	C	Walne, 1966
Crassostrea gigas	79–281	0.07–1.1[c]	0.4 – 4.4	25	2.82	0.96	C	Gerdes, 1983b
Ostrea edulis	180–297	0.17–0.27	0.5 –16.44[e]	n.s.	0.49	1.74	D	Holland & Spencer, 1973
Martesia cuneiformis	163–260	0.31–0.95	0.13– 1.96	22	[f]	[f]	D	Mann & Gallager, 1984
Teredo navalis	89–209	0.29–1.59	0.17– 1.86	23	0.98	1.24	D	This study
Bankia gouldi	68–230	0.17–2.2	0.18– 1.52	23	1.81	1.25	D	This study

[a] Dry tissue weight only estimated from length (here recalculated) by the relationship $W(\mu g) = 2.53 \times 10^{-9} \cdot L(\mu m)^{3.49}$ (Jesperson & Olsen, 1982).
[b] Value estimated from Holland & Spencer (1973) for a 48-h starvation period.
[c] Dry tissue weight only estimated as 25% of dry weight inclusive of shell (Gerdes, 1983a).
[d] Calculated from Walne (1966) Fig. 23A, first 10 h of experiment, value in text of Walne (1966) is not in agreement with Figure.
[e] Values calculated from Holland & Spencer (1973), Table 5 using the caloric equivalents reported in Crisp (1971).
[f] Insufficient values to calculate constant.

oxygen consumption illustrated by the data in Table IV also supports the suggestion that respiration rates do not change significantly during a starvation period of 48–72 h, although this remains to be axamined critically, and that the contribution of anaerobic processes to energy production is minimal in bivalve larvae. This latter point is in contrast to adult bivalves which have been shown in recent studies by De Zwaan *et al.* (1981), Famme *et al.* (1981), and Famme & Kofoed (1982) to have both aerobic and anaerobic components to their energy metabolism at all oxygen partial pressures.

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APPENDIX

Procedures for assay of protein, lipid and carbohydrate in bivalve larvae.

Homogenize or sonify 1–2 mg dry tissue or equivalent wet weight in 1000 µl H_2O.

300 µl: lipid assay

(1) + 100 µl H_2O + 1.5 ml 1:2 v/v $CHCl_3 : CH_3OH$
(2) shake (Vortex mixer) or sonicate
(3) stand 10 min
(4) centrifuge ≈ 1000 *g* for 10 min
(5) remove supernatant to a screw cap graduated tube
(6) to precipitate from (5) add 1.5 ml 2:1 v/v $CHCl_3 : CH_3OH$
(7) shake (Vortex mixer) or sonicate
(8) stand 10 min
(9) Centrifuge ≈ 1000 *g* for 10 min
(10) remove supernatant and pool with supernatant from (5) above
(11) add 950 µl of 0.7% w/v NaCl solution to pooled supernatants
(12) mix thoroughly, stand at 4 °C for > 30 min
(13) centrifuge ≈ 500 *g* for 10 min
(14) bottom layer ($CHCl_3$) contains lipid
(15) take known aliquots and dry for gravimetric assay
or
(16) take 1000 µl of bottom layer ($CHCl_3$) which contains lipid, dry at 60 °C
(17) + 500 µl H_2SO_4, mix gently
(18) heat to 200 °C for 15 min
(19) cool in water and ice bath
(20) add 2.5 ml water, mix, cool again, wait for bubbles to disperse
(21) read at 375 nm versus H_2O

(a) calibration versus cholesterol or tripalmitin dissolved in 2:1 v/v $CH_3OH : CHCl_3$ at (1) above
(b) reagent blank: solvents only at (1) above

500 µl: carbohydrate and protein assay

(1) + 250 µl cold 15% w/v trichloroacetic acid (T.C.A.)
(2) shake,
(3) stand > 10 min (preferably overnight) at 4 °C
(4) centrifuge ≈ 1000 *g* for 10 min

supernatant: carbohydrate assay

(5) 500 µl supernatant, + 500 µl H_2O + 500 µl 5% w/v phenol, mix
(6) + 2.5 ml H_2SO_4, mix gently
(7) stand > 20 min
(8) read at 490 nm versus H_2O

(a) calibration versus glucose dissolved in 5% w/v T.C.A. at (1) above
(b) reagent blank: solvents only at (1) above

precipitate: protein assay

(5) make to 1 ml with H_2O, mix
(6) + 5 ml reagent C (see below), mix, stand 10 min at room temperature
(7) + 500 µl 1 N Folin Reagent, mix, stand 30 min at room temperature
(8) read at 750 nm versus H_2O

(a) Lowry reagents:
A: 2% w/v Na_2CO_3 in 0.1 N NaOH
B: 0.5% w/v $CuSO_4$ in 1% w/v $KNaC_4H_4O_64H_2O$. Make fresh daily by mixing stocks 1:1 of 1% $CuSO_4$ and 2% $KNaC_4H_4O_64H_2O$
C: make fresh daily by mixing 50 ml A + 1.0 ml B
Folin Reagent is usually purchased at 2 N, dilute to 1 N
(b) calibration versus bovine serum albumen dissolved in H_2O at (5) above
(c) reagent blank: H_2O at (5) above

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