

Physiological characteristics of lipid-rich “fat” and lipid-poor “thin” morphotypes of individual *Calanus finmarchicus* C5 copepodites in nearshore Gulf of Maine

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Abstract

Calanus finmarchicus C5 copepodites may be found in a range of body morphologies from small, lipid-poor (“thin”) individuals to larger, lipid-rich (“fat”) individuals, which are often differentiated by depth. In order to assess the physiological status of these animals, C5s collected from nearshore Gulf of Maine were visually sorted by these criteria and assayed for activity of four enzymes characteristic of different physiological processes: citrate synthase (CS, Krebs cycle); glutamate dehydrogenase (GDH, protein catabolism); hydroxyacyl CoA dehydrogenase (HOAD, fatty acid catabolism); and laminarinase (digestive enzyme), as well as protein content. The lipid content of a smaller subset of animals was determined by image analysis of the lipid storage sac. Visual differences among copepods were reflected in their physiological characteristics. Thin C5s had less protein and lipid, and higher citrate synthase and laminarinase activity, than fat C5s. There was no difference in GDH or HOAD activity between the two groups. However, in both groups HOAD was negatively correlated with laminarinase activity, indicating that digestive activity is inversely related to capacity for β -oxidation of lipids. In contrast, *C. finmarchicus* C5s collected from 200 m in the Gulf of Maine as well as those raised in tanks had lower CS and laminarinase activity than the nearshore C5s, whereas HOAD activity was comparable with that of the fat nearshore C5s. These results suggest that metabolic responses are being induced in C5s advected into nearshore waters.

Calanus finmarchicus is a dominant component of the zooplankton in the North Atlantic. As with many members of the genus *Calanus*, the life cycle of *C. finmarchicus* includes an overwintering stage characterized by high levels of stored lipids and reduced metabolic expenditures during the C5 copepodite stage, resembling in many respects the diapause stage of insects (reviewed in Hirche 1996). In the Gulf of Maine, *C. finmarchicus* typically goes through two generations beginning in late December–January when overwintering C5s mature to adulthood and ending in the summer months as the C5 copepodites enter dormancy and sink to deep (300–400 m) waters (Meise and O’Reilly 1996; Miller et al. 2000).

Overwintering *C. finmarchicus* C5s display distinct physiological characteristics. Activity levels are low and oxygen consumption is reduced (Hirche 1983; Ingvarsdóttir et al. 1999), lowering overall energy demand. During overwintering the digestive system is greatly reduced, with reduced gut epithelium and low digestive enzyme activities (Hallberg and Hirche 1980; Hirche 1983). Lipid storage is pronounced and may account for as much as 50% of body volume (Miller et al. 2000).

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Although many *C. finmarchicus* C5s display characteristics that would normally be associated with an overwintering condition (e.g., torpor, large lipid sacs, reduced metabolism), individuals displaying normal activity patterns are often observed at the same time, generally segregated by depth. Active C5s tend to be smaller, with greatly reduced lipid sacs and elevated respiratory and digestive enzyme activities (Hirche 1983; Miller et al. 2000). Two populations may be observed: a deep, dormant population and a shallow, active one (Hirche 1983; Miller et al. 2000). In shallow waters of the Gulf of Maine (i.e., <100 m), the dormant individuals are unable to separate from the active and are found mixed together (Miller et al. 2000).

Even among visually identical C5s, different activity patterns may be observed. In the Gulf of Maine in November, C5s of the same size and appearance were observed in both a quiescent state at depth and in an actively feeding state in surface (0–80 m) waters (Durbin et al. 1997). Durbin et al. (1997) suggest that the active C5s had been advected from the bottom waters, where they were in dormancy, and reinitiated development in response to some cue. Deep-dwelling, lipid-rich C5s also vary in responsiveness regionally, with deep-dwelling individuals sometimes being unresponsive to physical prodding (Hirche 1983), whereas outwardly similar individuals from other locales, including the Gulf of Maine, may be very responsive (Miller et al. 1991). Variability in composition among individuals from a given location can be extreme, with four- to fivefold differences, or more, in dry weight and protein being observed within a population (Båmstedt 1988).

Faced with the variability in behavior and physiology of *C. finmarchicus* C5s, it is critical to understand the animal at the level of the individual copepod. Individual variability in copepod physiology and feeding behavior has been recognized as being a potentially significant part of the ecology of copepods and may reflect differing nutritional histories

within a population (e.g., Båmstedt 1988; Paffenhöfer 1993; Miller et al. 2000). Analyzing the physiology of *C. finmarchicus* C5s at the level of the individual can potentially shed light on how these animals are responding to their environment and can complement the integrative nature of data on body composition (e.g., body size, lipid, protein).

In order to determine whether active and dormant *C. finmarchicus* C5s could be distinguished by metabolic rates, and to determine variability of rates within the two groupings, activities of four enzymes (citrate synthase, glutamate dehydrogenase [GDH], hydroxyacyl CoA dehydrogenase [HOAD], and laminarinase) representative of important physiological processes were determined on individual *C. finmarchicus* C5 copepodites. Copepods were sorted based on readily distinguishable characteristics (relatively torpid individuals with large lipid sacs and responsive individuals with small lipid sacs). Citrate synthase, a key enzyme of the Krebs cycle, has been used as an indicator of nutritional status in copepods, as activity declines upon food deprivation (Clarke and Walsh 1993). GDH is involved in the deamination of amino acids and correlates with ammonia excretion in crustaceans (Park et al. 1986). HOAD is an enzyme involved in the β -oxidation of fatty acids, oxidizing hydroxyacyl CoA to β -ketoacyl CoA, and exhibits high levels of activity in *C. finmarchicus* (present study). Laminarinase (β -1,3-glucanase) is a digestive enzyme that hydrolyzes the polysaccharide β -1,3-glucan, a carbohydrate storage product of diatoms, and varies over a wide range seasonally in copepods (e.g., Hassett and Landry 1990). The nearshore animals were compared with a sample of *C. finmarchicus* C5 collected from deep water of Wilkinson Basin in the Gulf of Maine and a second group of C5s, visually identified as "fats," that had been raised from eggs in a large flow-through tank.

Materials and methods

Nearshore copepods were collected off the mouth of the Damariscotta River with a 100-m surface oblique haul using a 350 μ m mesh, 0.75 m diameter plankton net. *C. finmarchicus* C5 copepodites were sorted at the Darling Marine Center according to appearance as either fats (relatively torpid, large lipid sac) or thins (small lipid sac, more active). Twenty-three copepods from each category were sorted into beakers and the contents poured through 73 μ m mesh nitex filter to isolate the copepods. The filter was blotted from underneath with Kimwipes to remove excess water, and the animals were collected and added to cryovials, which were then frozen and stored in liquid nitrogen until ready for analysis. Representative specimens were also photographed under light microscope and the images were digitized and dimensions quantified using ImageJ (v.1.3.2) software (National Institute of Health). Calculations are based on percentage of total prosome volume, using the formula $V = \pi A^2/4L$, where A = x -sectional area and L = length of sac (Miller et al. 1998). An additional sample was collected by MOCNESS net from deep water (160–200 m) in Wilkinson Basin, Gulf of Maine, on 17 October 1999 (RV Endeavor Cruise 330) as part of the US GLOBEC Georges Bank pro-

gram. A subsample from the net haul was concentrated on nylon mesh, blotted with Kimwipes through the mesh to remove excess water, and frozen in liquid nitrogen. Samples were stored in liquid nitrogen until analyzed for biochemical composition. A final sample was taken from a culture system at the Darling Marine Center that utilized a 590-liter tank supplied with continuous-flow chilled seawater filtered through 100 μ m mesh. *C. finmarchicus* were raised from eggs under natural light cycle and ambient food supplemented with cultured dinoflagellates (*Prorocentrum micans*, *Gonyaulax polyedra*, and *Gymnodinium sanguinium*). All samples were stored in liquid nitrogen until analyzed for biochemical composition.

For biochemical analyses the frozen samples were placed on a microscope slide resting on an ice-filled Petri dish. Individual copepods were quickly identified by microscope if not previously sorted. Copepods were removed with forceps and placed in 1-mL tubes, also on ice. Next, 500 μ L homogenization buffer (40 mmol L⁻¹ HEPES {4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid}, 1 mmol L⁻¹ EDTA [ethylenediaminetetraacetic acid], and 2 mM MgCl₂ [pH 7.4 at 25°C]) was added to each tube and the sample was homogenized with three 10-s bursts using a 50 W ultrasonic processor with a 2 mm diameter tip. Generally, six copepods were individually sorted and sequentially homogenized at one time, with the homogenates held on ice until a total of 24 copepods had been prepared. The samples were then vortexed and subdivided for the biochemical assays using a multiple-tip pipetter. Enzyme assays were initiated immediately after subsampling was complete. Subsamples for protein were frozen for later analysis.

Citrate synthase activity was assayed by the fluorometric ABD-F method (Hassett and Crockett 2000) modified for use in a well plate reader (Perkin-Elmer LS50B spectrofluorometer equipped with a wellplate-reader attachment). Assays were conducted for 60 m at 23°C. Fluorescence intensities, indicative of CoA-SH binding to 7-fluorobenz-2-oxa-1,3-diazole-4-sulfonamide (ABD-F), were measured at 390/530 nm (excitation/emission wavelengths, respectively). A 430-nm cutoff filter was used and slit widths were set at 15 nm excitation/20 nm emission. Enzyme activities were measured in duplicate, with an additional subsample used as a blank. Reaction mixtures included 0.35 mmol L⁻¹ acetyl CoA in 50 mmol L⁻¹ HEPES (pH 7.8 at 25°C). Reactions were initiated with oxaloacetate (final concentration = 0.5 mmol L⁻¹). Parallel (blank) assays were run in the absence of oxaloacetate.

GDH and HOAD activity were determined following protocols in Passonneau and Lowry (1992). GDH was determined in a reaction mixture of 50 mmol L⁻¹ imidazole buffer pH 7.4, 25 mmol L⁻¹ ammonium acetate, 100 μ mol L⁻¹ adenosine phosphate (ADP), 50 μ mol L⁻¹ β -nicotinamide adenine dinucleotide phosphate reduced sodium salt (NADPH), 0.07% bovine serum albumin (BSA), and 2.0 mmol L⁻¹ α -ketoglutarate, Na salt. HOAD was determined in a reaction mixture of 50 mmol L⁻¹ imidazole buffer pH 6.1, 100 μ mol L⁻¹ β -nicotinamide adenine dinucleotide reduced sodium salt (NADH), 1 mmol L⁻¹ EDTA, 0.05% BSA, and 160 μ mol L⁻¹ acetoacetyl CoA. GDH assays were incubated for 90 min and HOAD assays for 60 min at 23°C.

Laminarinase (β -1,3-glucanase) was assayed following Hassett and Landry (1982), measuring glucose released during a 90 min incubation at 23°C. The assay was terminated by addition of 20 μ L 0.1 mmol L⁻¹ HCl, and the tubes were frozen for later fluorometric analysis of glucose (Passonneau and Lowry 1992). Protein was measured by the bicinchoninic acid method (Smith et al. 1985) using a Biotek microplate reader. HOAD, GDH, laminarinase, and protein assays were all run in triplicate. Blanks proved unnecessary for HOAD due to the high activity that was present, whereas blank fluorescence for GDH and laminarinase were largely due to the contribution of the reagent and not the sample. Only for CS activity was there significant contribution of the sample to blank values.

ABD-F was obtained from Dojindo Molecular Technologies, and Micro-BCA protein assay kit from Pierce Chemical Co., whereas all other biochemicals and other reagents were obtained from Sigma Chemical Co. All enzyme activities are expressed as units mg-protein⁻¹ (unit = μ mol substrate converted to product per minute).

Statistical analysis—Statistical analyses were performed with SPSS statistical software. Data are given as means \pm 95% confidence interval. Differences between means among the four groups were tested with the nonparametric Kruskal-Wallis test, and groups were then ranked using the Wilcoxon two-sample test. Partial correlation analyses were performed on log-transformed data, controlling for protein (which corresponds to animal size) within each group. Data are given in figures as mean \pm 95% confidence interval.

Results

Analysis of digital images of fat and thin *C. finmarchicus* C5s indicate that lipid content of the fat C5 (lipid sac = 23% \pm 3% of total body volume, $n = 6$) is more than five-fold greater than that of the thin C5s (lipid sac = 3.6% \pm 0.6 % of total body volume, $n = 4$) when comparing two-dimensional views of the copepods. In addition to higher inferred lipid content, fat C5s also had 1.6-fold higher protein content (Fig. 1A). Note that these values reflect the condition of the animals sorted by visual cues, which emphasized differences between “fat” and thin C5s, and do not necessarily reflect the distribution of lipid sac sizes in the field where intermediate forms may occur.

Enzyme activities of thin *C. finmarchicus* C5s were characterized by 1.2-fold higher citrate synthase activity (Fig. 1B) and 2.1-fold higher laminarinase activity (Fig. 1C) compared with fat C5s. Although a similar trend in higher activities for thin C5s is seen for HOAD and GDH activities (1.4- and 1.3-fold, respectively), the differences are not significant, obscured by high variability among individuals (Fig. 1D,E). HOAD activity was high, comparable with that of CS, whereas GDH activity was low, and in the presence of high reagent blanks was often difficult to detect.

C. finmarchicus C5s collected offshore were larger in size, based on protein content, than the fat C5s from nearshore or raised in tanks (Fig. 1A). Offshore specimens were frozen directly from the net haul so that a visual differentiation between fat and thin C5s was not possible. Thin C5s, how-

ever, have low protein content (Fig. 1A), so that the offshore animals, which had the highest protein content of the four sampled groups, are likely dominated by fat morphs, as would be expected from specimens collected from 160–200 m in the Gulf of Maine. Although the offshore animals had comparable HOAD activity to the nearshore and tank-raised fat C5s (Fig. 1C), both CS and laminarinase activity were significantly lower (Fig. 1B,E). GDH activity was not measured in the offshore or tank-raised groups.

Variability among individuals falls into two distinct patterns. Coefficient of variation (CV) was relatively low for CS and protein (a range of 16–26%, Table 1) and about three- to fourfold higher for GDH, laminarinase, and HOAD (a range of 58–92%), with both fat and thin copepodites displaying the same pattern. CV for image analysis of lipid sac volume also indicates low variability among fat or thin animals, albeit on different individuals than those used for enzyme activity measurements. High GDH variability may in part be attributable to the low activity observed relative to the blank value, which would lead to increased scatter in the data. However, both laminarinase and HOAD activities were detected over a broad range, with some activities being very high; HOAD in particular was always easily measured in individual copepods. Note that individuals were selected to conform to fat and thin characteristics and intermediate individuals would not be selected. Thus, the relatively low variability for lipid and protein content within each group is expected.

The strongest association among enzymes is that between HOAD and laminarinase activity among the nearshore copepods (Table 2, Fig. 2A). For both fat and thin C5s, HOAD activity was negatively correlated with laminarinase activity, indicating that HOAD was more prominent in copepods displaying low digestive activity, and conversely HOAD activity was low in those copepods with more active guts. CS and GDH activities were marginally correlated ($p = 0.059$) among thin CVs, and CS and laminarinase activities were marginally correlated ($p = 0.052$) among fat CVs. There was no association between HOAD and laminarinase among the copepods collected offshore (which were not sorted to fat or thin morphotype) or the fat C5s raised in the culture system (Fig. 2B).

Discussion

The results reported here indicate that visual differences among C5s are reflected in their metabolic capacities. Thin C5s are more responsive to handling and have significantly higher citrate synthase and digestive laminarinase activities than do the fat C5s. These results are similar to those of Hirche (1983) both in terms of the behavior of the animals (relatively torpid, lipid-rich C5s and active, lipid-poor C5s) and their metabolic potential. Sections of fixed copepods indicated that fat C5s had reduced gut epithelia and no developed B-cells, whereas thin C5s had a highly developed gut with large B-cells (P. Blades-Eckelbarger unpubl. data), consistent with the overall trends in the digestive enzyme data. B-cells are associated with digestive activity in copepods and are not present in overwintering *Calanus* (Hallberg and Hir-

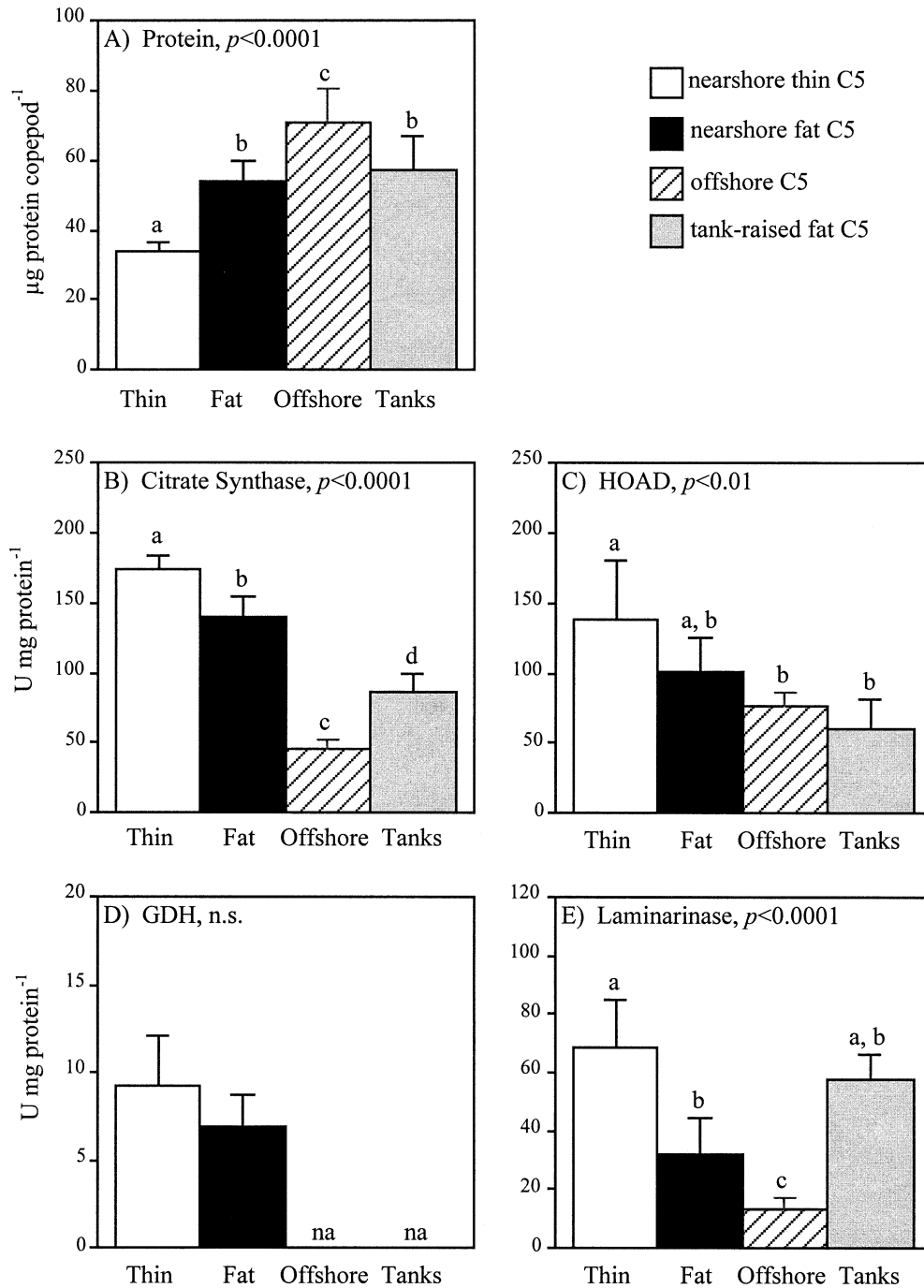


Fig. 1. Protein content and protein-specific enzyme activities of *C. finmarchicus* C5 collected nearshore (both fat and thin morphs), in deep water offshore (morphs not distinguished), and raised from eggs in large-volume tanks (fat morph). (A) protein; (B) citrate synthase; (C) HOAD; (D) GD; and (E) laminarinase (β -1,3-glucanase). Significant differences among means were determined with the Kruskal-Wallis nonparametric test and were indicated on the figure, and groups were then ranked with Wilcoxon two-sample test. Letters above columns denote significant differences between samples. Where two letters are present, sample could not be distinguished from either group. n.s., nonsignificant difference; na, data not collected.

che 1980). The relatively small number of individuals that could be analyzed by fixation and sectioning, however, makes it difficult to determine if a small percentage of the population had anatomical evidence of active guts, such as

the presence of B-cells, a pattern that would be suggested by the enzyme data in Fig. 2 in which a small number of fat C5s had relatively high digestive enzyme activity.

The frequently high activity of HOAD in *C. finmarchicus*

Table 1. *Calanus finmarchicus*. Coefficient of variation (%) among individual C5 copepodites collected in nearshore and offshore Gulf of Maine and raised in tanks. Value for lipid is derived from a different subset of animals from those used for the other data.

Measure	Nearshore fat	Nearshore thin	Offshore	Tank fat
Lipid (% total volume)	20	18	—	—
Protein	26	21	32	36
Citrate synthase	25	16	31	36
Hydroxyacyl CoA dehydrogenase	58	75	74	35
Glutamate dehydrogenase	64	74	—	—
Laminarinase	92	60	33	76

was notable in this study. As mentioned previously, HOAD is involved in the β -oxidation of fatty acids. The negative correlation with laminarinase in C5s indicates that this enzyme is present in copepods whose digestive system is less active and who likely are relying on stored lipids for metabolic needs. Conversely, when the digestive system is more active, the capacity for lipid catabolism is reduced. The observed correlation also suggests that even within a group (e.g., fat C5s), there may be considerable variation in metabolic strategy, with individuals being in various stages of slowing, or speeding up, their metabolic machinery. One factor potentially altering HOAD activity in dormant C5s is gonad maturation, a process that results in considerable depletion of lipid reserves (e.g., Gatten et al. 1980; Tande 1982). The onset of gonad maturation in the absence of an active gut would signal the start of higher rates of lipid catabolism and so would likely require higher HOAD activities. Alternatively, if the nearshore C5s were undergoing arousal from a diapause-like state after advection from deep waters (e.g., Pedersen et al. 1995; Durbin et al. 1997), higher lipid catabolism may be required in the short term to allow redevelopment of the gut cells, which deteriorate during dormancy (Hallberg and Hirche 1980). Neither the instantaneous picture provided by enzyme activities nor the integrative picture provided by lipid/protein composition is adequate to resolve this question.

The relatively low CV among nearshore copepods for citrate synthase, protein, and lipid within each category (approximately 20%) contrasts sharply with the high CV observed for laminarinase, GDH, and HOAD (50–80%). High

variability was only observed for HOAD activity among the tank-raised C5s. The high CV for laminarinase activity among offshore copepods is likely compounded by the very low activity of laminarinase in these copepods. Citrate synthase declines during starvation in *Acartia tonsa* (Clarke and Walsh 1993) and may correlate with the overall nutritional state of the copepod. Proteins and lipids would also be expected to similarly reflect nutritional history. The activities of HOAD, GDH, and laminarinase, while also perhaps reflecting nutritional history, may have an additional component to variability superimposed as individuals shift their metabolism in response to a change in environment (for instance, advection from deep water to nearshore). High variability is often observed in both gut fullness (e.g., Kleppel et al. 1988) and egg production rates (Båmstedt 1988), and may reflect the lack of synchrony among individuals in feeding activity (Båmstedt 1988).

Differences in energy sources (i.e., protein vs. lipid) between fat and thin C5s would be expected to lead to different O:N ratios, which relate oxygen consumed to nitrogen excreted (Mayzaud and Conover 1988). Because CS is involved in aerobic pathways and GDH in nitrogen excretion, CS/GDH may serve as a proxy for O:N ratio and thus energy utilization by fat and thin C5s. Precise determination of O:N would require calibration of CS and GDH activity for oxygen consumption and ammonia excretion, respectively, but for comparative purposes the ratio of the activities is sufficient. Also, because GDH activity was sometimes undetectable, calculating mean CS/GDH for these individuals ($n = 6$ out of a total of 46 individuals) becomes problematic, so median values were used. Median values of CS/GDH for individual fat and thin C5s are 16.4 and 18.8, respectively, whereas CS/GDH based on means reported in Fig. 1 are 19.4 and 20.3 for fat and thin C5s, respectively. Thus, based on CS/GDH there is no evidence that different sources of energy are being utilized between fat and thin C5s.

The development of *C. finmarchicus* in the Gulf of Maine in relation to oil sac fullness and the appearance of large and small (equivalent to fat and thin) C5s has been described by Miller et al. (2000). The initial G0 generation matures from dormant C5s beginning in late December. The first G1 generation reaches the C5 stage in March, with some descending to depth in April and others maturing to the G2 generation. This generation subsequently begins reaching C5 in June (when the sampling of Miller et al. 2000 ended). Because females continue to be present in the Gulf of Maine through the summer, the pattern of some C5s descending to deep water while others remain in shallow water and mature

Table 2. *Calanus finmarchicus*. Partial correlation coefficients for metabolic enzymes citrate synthase (CS), hydroxyacyl CoA dehydrogenase (HOAD), and glutamate dehydrogenase (GDH) as well as the digestive enzyme laminarinase (LAM), controlling for protein (animal size) within each group; r = partial correlation coefficient, p = level of significance. Degrees of freedom = 20. Correlations significant at >0.05 are highlighted in bold type. Activities were log-transformed for the analysis.

Comparisons	Thin C5s		Fat C5s	
	r	p	r	p
CS-HOAD	0.255	0.264	-0.241	0.280
CS-GDH	0.418	0.059	0.251	0.259
CS-LAM	-0.025	0.915	0.419	0.052
HOAD-GDH	-0.057	0.807	0.340	0.122
HOAD-LAM	-0.524	0.015	-0.591	0.004
GDH-LAM	0.084	0.717	-0.024	0.915

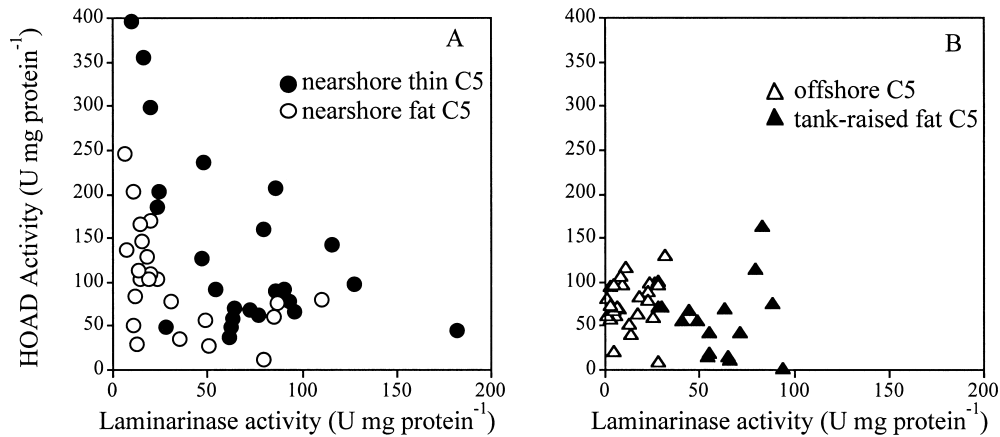


Fig. 2. *C. finmarchicus*. HOAD activity versus laminarinase activity of (A) nearshore thin and fat C5s and (B) offshore C5s and tank-raised fat C5s. HOAD and laminarinase were significantly negatively correlated in both nearshore thin C5s ($r = 0.57$, $p < 0.01$) and fat C5s ($r = 0.50$, $p < 0.01$), but were not significantly correlated in either the offshore C5s or tank-raised C5s.

to adulthood likely repeats itself in the G2 generation. This pattern is consistent with Clarke's (1933) survey in the Gulf of Maine during July, when *C. finmarchicus* C4 and C5 had a maximum abundance in deep water (≈ 100 – 120 m), but still had significant numbers near the surface. Even in November, a significant fraction of the *C. finmarchicus* population may be present in surface waters (0–80 m) in the Gulf of Maine (Durbin et al. 1997). In the latter study, surface C5s were identical in terms of body mass and appearance to those at depth, but were actively feeding as well as apparently vertically migrating. Durbin et al. (1997) speculate that advection by bottom currents carried some C5s to the surface, where they renewed development. This hypothesis would be consistent with the observed distribution of HOAD and laminarinase activities among fat C5s, with some animals shifting their metabolism to a more active pattern.

Although thin and fat C5s have distinct physiological differences, the question remains as to whether thin C5s will over time accumulate lipid reserves and take on the features of the fat C5s, or whether they represent an alternate developmental pathway. Miller et al. (2000) attribute the short (= thin) C5s in the surface layer in June to the G2 generation and speculate that these C5s will accumulate lipids and descend in late June/July. However, studies of *C. finmarchicus* development in mesocosms demonstrated that a fraction of the C5s molted to a G2 generation while the remainder continued into diapause (Marker et al. 2003). They point to a "continuum of physiological endpoints" for *C. finmarchicus* C5s, with a range of individuals either preparing for diapause or preparing to molt to adult. The proposal of Rey-Rassat et al. (2002) of a threshold value for lipid accumulation to initiate diapause would provide the cue for which direction to go. Although improved feeding conditions might allow the lipid-poor C5s to acquire the necessary lipids to undergo diapause, at some point during development a decision must be made to proceed to molting, and additional lipid deposition beyond that point presumably would not alter that pathway. Thus, C5s may have alternate developmental pathways depending upon feeding history.

Under such a scenario the response of *C. finmarchicus* to low food conditions would be critical in controlling the appearance of thin C5s. Campbell et al. (2001) observed a strong effect of food limitation on lipid storage of *C. finmarchicus* raised in mesocosms, noting that carbon growth rates were reduced much more than nitrogen growth rates during food limitation. Adult female *C. finmarchicus* delay gonad maturation when presented with low food conditions (Hirche 1996), which also points toward a similar central role for nutrition in development. Gonad maturation, however, may be more advanced in thin than fat C5s collected from the field (P. Blades-Eckelbarger pers. comm.). Although these observations of gonad maturation are limited in number, they suggest that thin C5s are not simply food-limited (and therefore not yet depositing lipid reserves), but have put their limited resources into reproductive tissue at the expense of lipid storage, thus choosing an alternative strategy from the fat C5s who delay gonad maturation in favor of lipid accumulation.

Hind et al. (2000) suggest that what is commonly referred to as diapause in *C. finmarchicus* is a slowing of metabolic processes due to low food conditions, rather than a hormonally controlled process cued to external factors such as photoperiod. Advection of metabolically inactive fat C5s from bottom water in the Gulf of Maine into shallow, nearshore waters with more abundant food could result in induction of enzymatic activity, with individual responses varying depending on the animal's history (e.g., exposure to different water temperature and food levels). Induction of activity, however, could also occur if the copepods were aroused from a diapause-like state by advection to the surface, provided that control of diapause was not strictly regulated by photoperiod; therefore the results reported here cannot distinguish between the two hypotheses. If the C5 developmental pathway to diapause can be triggered by reaching a threshold amount of lipid reserve (Rey-Rassat et al. 2002), then a similar response may lead the C5s to descend to deep water, where low temperatures and low food availability could lead to a metabolic slowdown. In either case, the thin C5s may

represent animals trapped in a direct development path, putting the little available resources into reproductive tissue while preparing for the terminal molt.

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