# Daily carbon, nitrogen and phosphorus budgets for the Mediterranean planktivorous damselfish *Chromis chromis*

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## Abstract

The planktivorous damselfish *Chromis chromis* is one of the most numerous fish species in the Mediterranean rocky littoral, yet the role this species plays in the overall functioning of the ecosystem is poorly understood. In this study nitrogen, carbon and phosphorus budgets were constructed for *C. chromis* from the Bay of Calvi, Corsica and an attempt was made to resolve the possible fates of these nutrients from ingested materials, to somatic growth ($P_s$), reproductive tissues ($P_r$), respiration ($R$), assimilation ($A'$), faeces ($F$) and dissolved wastes ($U$). *C. chromis* were estimated to consume on average 4.7% of their own body mass of zooplankton per day, in two separate feeding episodes — at dawn and at dusk. This is equivalent to 8288 individual copepods or 42.1 mg carbon, 9.7 mg nitrogen, 0.8 g phosphorus. 44% of all phosphorus ingested was shown to be excreted as dissolved waste, and 46% as faeces; this may be of considerable importance in the Mediterranean, where littoral macrophytes are often severely phosphorus-limited. 85% of all nitrogen was excreted as dissolved waste (mostly ammonia) and local nutrient enrichment may enhance littoral primary productivity, particularly around *C. chromis* night-shelters.
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## 1. Introduction

In the Mediterranean rocky littoral, there is a general lack of data concerning planktophagous fish, despite planktivores representing 80–85% of the fish assemblage at some sites (Harmelin, 1990). The planktivorous damselfish *Chromis chromis* is one of the most nu-

merous species of this system (Harmelin, 1987; Spyker and van den Berghe, 1995), yet the role that this fish plays in the overall functioning of the ecosystem has never been studied in any great detail. During daylight *C. chromis* feed in large aggregations on the incoming plankton, but when night falls, these fish gather over the rocky littoral and settle into crevices among the rocks (Harmelin, 1987). In the process, large amounts of plankton-derived material is picked up as food offshore and released as faecal pellets directly onto the substratum (Pinnegar and Polunin, 2006). Densities of

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resting planktivorous fish may reach 5–10 individuals per square metre (Harmelin, 1987), consequently damselfish may act as important vectors, transferring nutrients and nanominerals from the pelagic to the littoral.

The waters of the Mediterranean are among the most oligotrophic described anywhere in the world (Krom et al., 1991). The western basin is particularly limited in nitrogen whereas the Adriatic and eastern basin appear to be depleted in phosphorous. Mediterranean macro-algae are particularly poor sources of these nutrients (Atkinson and Smith, 1983) and consequently it is not clear whether the diverse assemblages of fish and invertebrates which live in Mediterranean coastal areas are supported by pelagic (plankton) or benthic (algal) food chains.

The specific aims of this study were to quantify the amount of plankton consumed by individual *C. chromis* in spring, and also to calculate the amount of specific nutrients excreted (and thus imported to littoral food-webs) in the form of faeces and dissolved wastes. The study also aimed to resolve the possible fates of carbon, nitrogen and phosphorus from ingested materials, once they have entered the fish and their relative apportion-ment to production ($P$), respiration ($R$), assimilation ($A'$), faeces ($F$) and dissolved wastes ($U$).

There has been much work on the feeding and physi-ological energetics of territorial and herbivorous damsel-fish (e.g. Polunin and Brothers, 1989), but in comparison, few studies have focused on planktivorous damselfish, despite their abundance on tropical and temperate reefs. Mapstone and Wood (1975) reported on the social or-ganisation and behaviour of *C. chromis*, whilst the re-productive cycle has been described by Dorn et al. (1979), Contini and Donato (1973) and Dulčić and Kraljević (1994). Stomach-contents data have indicated that *C. chromis* feeds overwhelmingly on copepods, and other large plankters such as ostracods and larvaceans (e.g. Duka and Shevenko, 1988; Dulčić, 2007), whilst early work by Vernon (1895) yielded data on oxygen consumption. Here, we aim to produce balanced nutrient budgets for three important elements, namely nitrogen, carbon and phosphorus.

Nutrient or energy budgets for individual fish, usu-ally take the form:

$$C = P + R + F + U \tag{1}$$

where $C$ is the nutrient content in the food consumed over a defined time period, $P$ is the fraction of the resource which is used in the production of body tissues, $R$ is the fraction which is lost during metabolism (i.e. during respiration), $F$ is the fraction lost as faeces and $U$

is the remaining component lost in the form of excretory products (e.g. ammonia, dissolved phosphates) (Woo-ton, 1998). Production ($P$) can be further sub-divided:

$$P = P_s + P_r \tag{2}$$

where $P_s$ is the fraction of the resource directed towards somatic growth (structural and reserves) and $P_r$ is the fraction directed towards reproductive tissues (gametes). The fraction of resources which are absorbed ($A$), some-times called 'digestibility', can be obtained from the equation:

$$A = C - F \tag{3}$$

and the proportion of the absorbed resource which is assimilated ($A'$):

$$A' = A - (U + R) \tag{4}$$

## 2. Methods

This investigation was carried out at the STARESO research station Corsica, over five field seasons: April–May 1997, August–September 1997, March–June 1998, September 1998 and May–June 1999. Adult fish were collected using a small multi-pronged spear during the day, or using a 5% (in alcohol) solution of the anaesthetic quinaldine, which was squirted into night-shelters during the hours of darkness. Juvenile *C. chromis* were caught using hand-held aquarium nets during the day. Fish were either, (a) measured (standard length, total length, mass) and then released, (b) measured and used in respiration/excretion experiments, or (c) for those which had been speared, measured and gut, gonad, muscle tissues dis-sected out. Tissues were weighed and dried at 60 °C, before being transported to the laboratory for further analysis. Daily nutrient budgets were constructed for the spring period, because most field measurements, in par-ticular daily consumption estimates, were collected in this season.

### 2.1. Chemical analyses

Carbon and nitrogen content (%) were determined for dry tissues using a single inlet duel collector, mass spectrometer (Automated Nitrogen Carbon Analysis (ANCA) SL 20-20 System). Phosphorus concentration was determined by digesting the dry material in con-centrated $HNO_3$ for 1 week, diluting the sample to 15% acid with ultra pure water and then introducing the sample into a Unicam-701 series Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES). The

phosphorus standard was VHG Labs Inc. Plasma Emission Standard. All tissue samples were analysed in triplicate (from three different fish).

### 2.2. Production

Somatic growth ($P_s$), can be expressed according to the von Bertalanffy growth curve:

$$L_t = L_\infty \left(1 - e^{(-K(t-t_0))}\right) \tag{5}$$

where $L$ is fish total length at time $t$, $L_\infty$ is the asymptotic length, $k$ is the growth constant ('curvature parameter') and $t_0$ is the 'initial condition parameter' which defines the theoretical point in time when a fish has zero length. These parameters were estimated from length–frequency data using the Elefan method described by Pauly (1987) and Shepherd's Length Composition Analysis (SLCA), devised by Shepherd (1987). Length–frequency data were collected during all five field seasons but were based only on individuals caught at night using quinaldine or hand-netted during the day, since the inclusion of speared fish resulted in histograms disproportionately skewed towards larger fish. Measurements from a total of 892 individuals were incorporated, and dates were expressed as decimals of the Julian year (e.g. 1st January 1997=0.003, 1st January 1998=1.003).

The length–weight relationship (Eq. (6)) for the population was established using measurements on all 1146 fish collected throughout the three-year period.

$$W = aL^b \tag{6}$$

$W$ is wet mass (g) and $L$ is total length (cm). Differences between sexes were investigated for fish in May–June both in 1998 and 1999 prior to spawning. The relationship between fish wet mass and dry mass was measured in April/May 1998, based on ten fish, ranging in size from 1.28 to 22.14 g fresh mass. The carbon, nitrogen and phosphorus content of homogenised whole fish was used in order to calculate the proportion dedicated to somatic growth, although measurements were also taken of C, N, P content in dorsal white muscle, which represents the most abundant individual tissue in the body of fishes. Whole-ground juvenile fish (1.3–2.9 cm) were also analysed.

### 2.3. Reproductive growth ($P_r$)

The relationship between gonadal mass and fish wet mass was investigated for 24 males (3.6–33.8 g fresh weight) and 14 females (9.5–31.3 g fresh weight) in early

June 1999, when male fish had started to exhibit the first signs of display/courtship behaviour (Mapstone and Wood, 1975; Picciulin et al., 2004). Complete gonads were dissected out of freshly speared individuals, and weighed wet and dry, before being analysed for C:N:P content. During the day, adult males were greatly more abundant over *Posidonia oceanica* seagrass meadows (at ~16 m depth) than females. Males occasionally exhibited the characteristic 'jumping' courtship behaviour described by Mapstone and Wood (1975), and remained closer to the substratum, than was the case earlier in the year (April–May), making them easier to capture. Adult females, were comparatively rare during the day and were mostly caught at night from within their shelters, using quinaldine or spear. Daily estimates of gonadal growth, were based on the total weight of gonads in the period immediately prior to spawning (in June), divided by 182.5 days, since Contini and Donato (1973) suggested that *C. chromis* gonads take six months to develop, after a period of six months dormancy each year.

### 2.4. Consumption/ingestion

During May 1997 and May 1998, considerable effort was made to determine patterns in feeding periodicity of *C. chromis*. 164 fish (5.2–35.5 g) were speared at different times throughout the day (between 05:00 and 01:00), and their alimentary tracts were immediately removed (from mouth to anus) and weighed (wet mass). The weights of fish guts (including contents) were expressed as percentages of fish wet mass (WW), in order to compensate for any effects of size. The day was then divided into 10 two-hour time periods (no data were available from 01:00 to 05:00) and the mean gut fullness calculated for all fish caught during each time increment. A total of 16 live fish (6.5–20.4 g) were also captured and held in the laboratory for 68 h to ensure complete voiding of gut contents. This provided size-specific estimates of the mass of empty gut tract. Differences in gut mass among time periods were assessed using Student's $t$-test ($\alpha < 0.05$).

The relationship between the mass of empty alimentary tract (wet) and fish fresh mass, was best described ($r^2 = 0.977$) by the equation:

Empty alimentary tract $= (\text{fish WW} \times 0.02) + 0.01.$
$$\tag{7}$$

The mean mass of gut contents was calculated by subtracting the size-specific mass of empty gut from the total gut mass measured in the field. These results, expressed as a percentage of fish fresh mass (WW) were

then used to calculate daily ration ($R_d$) for the population. Sainsbury (1986) developed a method by which ingestion rate and evacuation rate could be calculated from stomach contents data. Following this approach, the International Centre for Living Aquatic Resource Management (ICLARM) developed a computer model, MAXIMS (Jarre et al., 1991), which computed the various parameters from field data through a process of non-linear regression. This method has been reviewed and refined by Richter et al. (1999), and it is this latest version which was used here. It was assumed that there are two feeding periods (see Fig. 1) and that the ingestion rate during the feeding period(s) was constant (model 2.1 in the terminology of Jarre-Teichmann et al., 1990; Richter et al., 1999). Richter et al. (1999) provided a series of routines for use with the statistical package SAS for windows (version 6.11 and higher), these require estimates for the ingestion rate ($J$), the instantaneous evacuation rate ($E$) (in this case equivalent to defecation rate), the time at which feeding begins ($T_r$) and the time at which feeding ends ($T_f$). In this study, however the ingestion rate ($J$) differed in the two feeding periods and was considerably slower in the afternoon than in the morning (Fig. 1). Consequently, a new version of the Richter et al. (1999) model 2.1 was created (H. Richter, personal communication) in order to allow for different values of $J$. Since the results are based on measurements for the whole alimentary tract, and not simply the stomach contents as in most MAXIMS applications, estimated evacuation times and peaks in gut fullness were almost certainly later in the day than
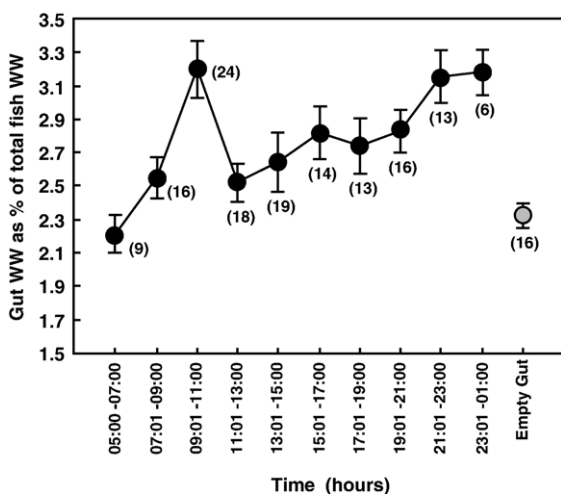


Fig. 1. Mean gut fullness throughout the day in the planktivorous damselfish *Chromis chromis*. Measurements are in % of fish body mass (including the mass of the alimentary tract), error bars are 1 standard error, numbers of individuals given in parentheses.

would be the case if stomach mass alone had been measured. As a consequence of this time lag, the peak in gut fullness following the afternoon feeding period occurred after dusk (between 20:30 and 21:30) and persisted for several hours until evacuation of the gut occurred via defecation. This (the second feeding phase apparently going past midnight) created problems mathematically, and thus for computation of daily ration ($R_d$), the timings of each data point and input parameter were shifted two hours earlier; this did not affect estimations of food consumed but allowed the SAS procedure for segmented non-linear regression to converge. Once estimated/modelled, parameters (J1, J2, $T_{r1}$, $T_{f1}$, $T_{r2}$, $T_{f2}$) had been obtained, daily ration ($R_d$) was estimated via:

$$R_d = J1 \times (T_{f1} - T_{r1}) + J2 \times (T_{f2} - T_{r2}). \tag{8}$$

Carbon and nitrogen absorption efficiencies calculated for this population of *C. chromis* are reported elsewhere (Pinnegar and Polunin, 2006), and were estimated for spring 1997 using the ash marker method and the equations of Montgomery and Gerking (1980). Absorption (digestibility) of phosphorus was estimated by mass balance, assuming that:

$$A = C - (U + P). \tag{9}$$

### 2.5. Assimilation and excretion

Excretion of total phosphorus was estimated by placing 5 freshly caught live fish (captured just after dusk using quinaldine), into individual 10 l, acid-washed aquaria, containing filtered seawater for 24 h and removing water samples (5 ml) at the beginning (0 h) and end (24 h) of the experiment. Samples were also taken from an identical, but empty aquarium in order to act as a control and to examine whether there was significant excretion of phosphorus by micro-organisms in the seawater over the 24 h period. Water samples were immediately frozen and 0.5 ml of concentrated HCl was added to preserve the sample during transit. The samples were analysed using a Unicam-701 series ICP-OES, as described earlier for the chemical analysis of tissues. Assimilation efficiency and thus excretion of nitrogen and carbon were calculated through mass balance, assuming that:

$$A' = P_s + P_r \tag{10}$$

and that

$$U = A - A' \tag{11}$$

or for carbon

$$U = A - (A' + R). \tag{12}$$

Table 1
Mean carbon, nitrogen and phosphorus composition (±1 standard error) of C. chromis tissues, faeces and plankton (% of dry mass) from the Bay of Calvi, Corsica measured in spring 1997 (plankton and faeces) and spring 1999 (all other tissues)

|  | Plankton | Whole Fish | Fish white muscle | Juvenile Chromis | Testes | Ovary | Faeces |
|---|---|---|---|---|---|---|---|
| % C | 34.99%±0.39 (3) | 50.48%±0.95 (3) | 45.41%±0.16 (3) | 38.25%±0.8 (3) | 45.13%±0.37 (3) | 52.80%±0.67 (3) | 13.35%±0.26 (3) |
| % N | 8.06%±0.14 (3) | 8.32%±0.15 (3) | 12.30%±0.21 (3) | 9.44%±0.21 (3) | 11.80%±0.40 (3) | 10.35%±0.14 (3) | 1.46%±0.01 (3) |
| % P | 0.68%±0.07 (3) | 2.19%±0.14 (3) | 0.94%±0.01 (3) | 2.59%±0.06 (3) | 2.12%±0.13 (3) | 1.06%±0.01 (3) | 1.73%±0.04 (3) |
| ∼C:N:P | 15:12:1 | 23:4:1 | 48:13:1 | 15:4:1 | 21:6:1 | 50:10:1 | 8:1:1 |

## 2.6. Respiration

Measurements of oxygen consumption in resting C. chromis were conducted in the laboratory at the University of Groningen (Netherlands) using fish transported from the STARESO research station. Fish were placed individually in a 2 l respirometry chamber, and water was passed through the chamber from a header tank of oxygen-saturated water (see van Rooij and Videler, 1996). Flow rate was measured at regular intervals and oxygen concentration measured in the outflowing water every 5 min, using a YSI 5750 palarographic oxygen electrode in a separate stirred chamber. Flow rate and oxygen concentration were used to calculate the quantity of oxygen ($O_2$) consumed by each fish, expressed as milligrammes of $O_2$ consumed per second. Five fish, ranging in size from 6.67 g to 14.85 g were used in these experiments, and the experimental duration ranged from 14 h 30 min to 24 h 10 min, with a period of darkness in each case, between 19:03 until 07:03 (12 h duration). Water temperature was maintained at a constant 20 °C, and the resulting data (expressed in mg $O_2$ consumed per gram of fish tissue per day) were thus adjusted to the mean water temperature for May in Corsica (16.9 °C), using the relationship:

$$\ln (O_2 \text{ consumed}) = (\text{temperature}(°C) \times 0.13) + -2.11 \tag{13}$$

derived from that reported by Vernon (1895). Values were then further converted to carbon (and $CO_2$) production assuming that for every one molecule of $O_2$ consumed, one molecule of $CO_2$ was produced, and that the molecular mass of oxygen was 16.0 and of carbon, 12.0.

## 3. Results

### 3.1. Elemental composition

The carbon and nitrogen content of plankton collected in the Bay of Calvi (Table 1), was significantly higher than is the case for faeces produced by C. chromis ($P = 4.0 \times 10^{-7}$ and $2.2 \times 10^{-4}$ respectively), indicating that these nutrients were selectively absorbed during their passage through the gut of this fish (see Pinnegar and Polunin, 2006). It appeared that proportionally less carbon (84%) was absorbed than nitrogen (93%) and paradoxically that significantly more phosphorus was egested in the faeces ($P = 2.2 \times 10^{-5}$), than was consumed. All body tissues (including whole adult or juvenile individuals) contained large amounts of carbon (Table 1), while white muscle and gonadal tissues also contained high quantities of nitrogen. Whole-ground adult and juvenile fish were most enriched in phosphorus, while testes contained significantly higher quantities of phosphorus than were present in other soft tissues ($P = 6.8 \times 10^{-3}$).

### 3.2. Production (P)

The measured length of the 1146 individual C. chromis ranged from 1.02 cm to 14.30 cm and the weight from 0.02 g to 42.15 g. Length–weight regressions were estimated separately for males and females and for all fish from all seasons combined (Table 2). The slopes of the length–weight regressions did not differ significantly between sexes.

Length–frequency distributions of fish collected over the four different field seasons (Fig. 2), allowed parameters of the von-Bertalanffy Growth Function to be calculated. In the autumnal sampling seasons (August–September), there were peaks in newly 'recruited' fish of less than 2 cm, which by the spring of the following year manifested themselves as discrete peaks between lengths of 4 and 6 cm. Both Elefan and SLCA clearly

Table 2
Parameters of the regression ($W = aL^b$) between total length (cm) and fresh weight (g) for C. chromis in the Bay of Calvi

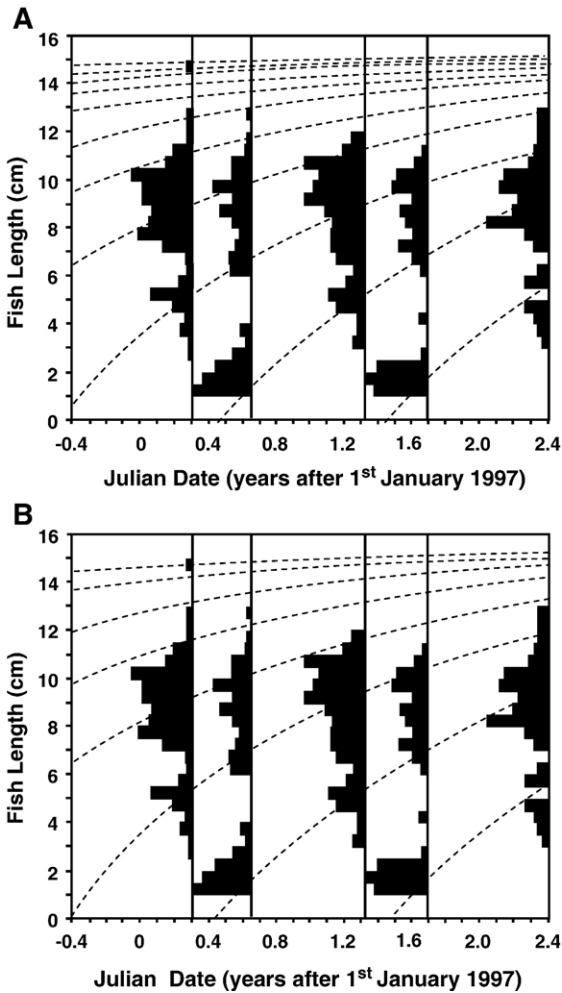| Sex | a | b | S.E. (b) | N | $r^2$ |
|---|---|---|---|---|---|
| Females | 0.019 | 2.96 | 0.22 | 19 | 0.92 |
| Males | 0.016 | 3.02 | 0.10 | 26 | 0.98 |
| All fish | 0.014 | 3.05 | 0.01 | 1146 | 0.99 |

Fig. 2. Von Bertalanffy growth curves calculated by Shepherd's Length Composition Analysis (A) and Elefan (B), superimposed over length frequency distributions for *C. chromis* in the Bay of Calvi, Corsica. Sampling dates (in Julian years) correspond with 20/4/97, 26/8/97, 29/4/98, 9/9/98, 26/5/99.

picked out this modal progression (Fig. 2a and b). However, distinct peaks in the distribution of larger fish were less apparent, and the two methods (Elefan & SLCA) differed in their interpretation of these larger size classes. Most larger fish, were indicated to be between 2 and 3 years of age, although the 14.3 cm fish caught in the first field season (20/4/97, i.e. date 0.301) was 5.67 years old according to the Elefan method, but 7.15 years old according to SLCA. The best fitting von-Bertalanffy Growth Function according to Elefan (score=0.528) was described by:

$$L_t = 15.78\left(1 - e^{(-0.464(t-0.570))}\right) \tag{14}$$

whilst SLCA (score=41.809) resulted in the expression:

$$L_t = 14.91\left(1 - e^{(-0.486(t-0.573))}\right). \tag{15}$$

These relationships were broadly similar to those obtained by Dulčić and Kraljević (1995) for *C. chromis* based on examination of scale rings. If the mean wet mass of adult fish in this population is taken as 10.25 g (the mean wet mass of fish based on the 338 caught by quinaldine in 1997) then the value obtained through SLCA and using the length–weight exponents, equates to an accrual of tissue over the course of a year of approximately 11.11 g or 0.03 g per calendar day.

The relationship between fish wet mass ($x$) and fish dry mass ($y$) was best described ($r^2=0.994$) by the regression:

$$y = 0.31x + 0.07 \tag{16}$$

and consequently, a 10.25 g fish would grow by 3.51 g dry mass per year (10 mg per calendar day), and allocate carbon, nitrogen and phosphorus to this function in accordance with the C:N:P ratio of 'whole fish' in Table 1.

Fig. 3a indicates that gonadal growth ($P_r$) in female *C. chromis* followed an exponential trajectory which was best described ($r^2=0.742$) by the equation:

$$\ln y = (1.76 \times \ln x) - 4.37 \tag{17}$$

where $x$ is fish fresh mass and $y$ is the combined wet mass of both ovaries. If we assume that ovary dry mass represented 31.5% of ovary wet mass (J.K.Pinnegar, unpublished data), that *C. chromis* ovaries were greatly reduced after each spawning event, and thus that the females 're-grew' large ovaries prior to spawning each year (Contini and Donato, 1973) then a 10.25 g female produced approximately 0.24 g dry mass of gonad tissue over the six month period (1.3 mg on average, per day).

Fig. 3b indicates that gonadal growth ($P_r$) in male *C. chromis* followed an allometric course which was best described ($r^2=0.806$) by the equation:

$$y = (0.55 \times \ln x) - 0.65 \tag{18}$$

where $x$ is fish fresh mass and $y$ is the combined wet mass of both testes. Male fish dedicated lower quantities of nitrogen, carbon and phosphorus to reproductive growth, although producing many millions more gametes. Assuming that testicular dry mass represents 19.07% of testes wet mass (J.K. Pinnegar, unpublished data), and that after each spawning event *C. chromis* testes were also greatly reduced (Contini and Donato,
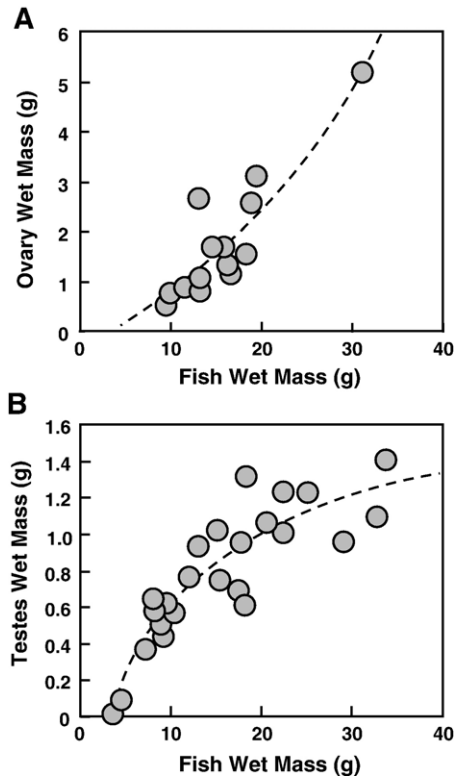
Fig. 3. Relationships between female (A) and male (B) gonad mass (wet mass) and total body mass (in grams) in the planktivorous damselfish *Chromis chromis*.

1973) then a 10.25 g male would produce approximately 0.120 g dry mass of gonad tissue in the six month period prior to spawning (0.7 mg/day).

### 3.3. Consumption

Gut mass/fullness data (Fig. 1) exhibited great variability between individual fish, but it appears that fish began feeding rapidly soon after dawn (around 05:00 h in April/May) and individuals reached gut fullness between 09:00 and 10:00. Close examination of stomach contents and faeces during this period revealed copepod exoskeletons (in particular *Clausocalanus arcuicornis* and *Centropagis typicus*) and occasionally the shells of pteropod molluscs, thus confirming that diet did closely match the zooplankton available in the local environment (Dauby, 1980) and also largely agree with Dulčić (2007). Gut masses (as a % of fish mass) were significantly higher in the 9:00–11:00 time period than in the 5:00–7:00 time period ($p = 0.00004$). Consequently it seemed to take only 4–5 h of feeding for the gut to become full, and this equates to approximately 0.21 g (wet mass) of material. Assuming

that the average fresh mass of the dominant calanoid copepod species (*C. arcuicornis*) in the waters of the Bay of Calvi was $0.058 \pm 0.003$ mg (SD) (J.K. Pinnegar, unpublished data), then as many as 3638 individual food items were consumed before 10:00. Following this initial peak, gut mass declined (Fig. 1) to a level which was not significantly different from that of empty tract ($p = 0.305$), but then increased slowly throughout the afternoon and more rapidly around dusk (19:00–21:00). Guts were significantly heavier between 23:00–01:00 than in the early morning (05:00–07:00) ($p = 0.001$) and also heavier than guts of fish starved in the laboratory for 62 h ($p = 0.001$). The mass of alimentary tracts from fish captured in early morning were not significantly different from those of laboratory-starved fish, indicating that *C. chromis* start the day with empty guts and have presumably deposited the contents from the night before into their night-shelters. Using the MAXIMS input parameters $J_1 = 0.6$, $J_2 = 0.25$, $E = 0.4$, $T_{r1} = 5.5$, $T_{f1} = 8.1$, $T_{r2} = 11.3$, $T_{f2} = 22.2$ (all times shifted earlier by two hours), it was possible to calculate daily ration ($R_d$). The corresponding modelled parameters were $J_1 = 0.620$, $J_2 = 0.327$, $E = 0.447$, $T_{r1} = 5.475$, $T_{f1} = 8.077$, $T_{r2} = 11.265$, $T_{f2} = 22.000$ and thus we estimated a daily ration of 4.69% of body mass per day (1.41% in the first feeding phase, 3.27% in the second). For a 10.25 g fish this equated to 0.48 g of plankton consumed each day, or 0.12 g dry.

### 3.4. Excretion

Measurements of total phosphorus concentration in the empty control tank, prior to and after the 24 h experimental period, were not significantly different ($p = 0.402$), but in all other tanks containing fish, total phosphorus content was significantly higher in the water after 24 h, indicating that the fish had excreted phosphorus. The amount of phosphorus excreted increased linearly with the size (mass) of the fish, and was best described ($r^2 = 0.926$) by the equation:

$$P_{24} = (M \times 0.05) + 0.32 \qquad (21)$$

whereby $P_{24}$ is the quantity of phosphorus produced (mg) over 24 h, and $M$ is fish fresh mass (g). Using this equation, we can conclude that a 10.25 g fish would excrete approximately 0.83 mg of phosphorus per day.

### 3.5. Respiration

Experimental fish generally exhibited similar patterns of oxygen consumption throughout the day, with

periods of elevated consumption shortly after the end of the simulated night-time period (Fig. 4). Using the data from all five fish, converted to mg, a mean rate of $1.68 \pm 0.39$ (SD) mg of $O_2$ consumed per gram of fish tissue per day was obtained, and this equated to 2.31 mg of $CO_2$ per gram of fish tissue per day or 0.82 mg of carbon per gram per day. When corrected to the ambient temperature in May 1998 (16.9 °C) this suggested an oxygen consumption for a 10.25 g fish of 15.01 mg of carbon per day.

## 4. Discussion

### 4.1. Elemental composition

Early work concerning the elemental composition of *Chromis* (*Heliastes*) *chromis* tissues (Milone, 1896) suggested that 3.18% of fresh mass was nitrogen, which corresponds to 12.69% nitrogen of dry weight and generally agrees with our observations. However, 1.67% was suggested to be phosphate ($P_2O_5$) (Milone, 1896) and this corresponded to a percentage of dry weight of 4.40% phosphorus, which is higher than the value reported here (Table 1). Vinogradov (1953) suggests that among the soft tissues, the largest amount of phosphorus is generally found in the gonads and particularly so in the testes, and this is also in agreement with the present observations for *C. chromis* soft tissues (Table 1). The higher values observed for whole adult and juvenile fish reflected the high content of inorganic phosphates in bones and scales. Vinogradov (1953) suggested that fish roe contains more carbon (∼50–56%) than other tissues, and this agrees with the high carbon content we observed for ovary tissue. The

highest proportion of nitrogen was observed in white muscle tissue, reflecting the high protein content of this material.

### 4.2. Consumption and feeding periodicity

The fish feeding model MAXIMS has been widely applied over the past decade, and the model has been shown to be mathematically robust when artificial variability was introduced into a theoretically perfect stomach-fullness data set (Richter et al., 1999). Our finding that *C. chromis* exhibited two peaks in feeding, one in the early morning (9–10 am) and one in the evening (∼9 pm), is unlike the pattern reported for herbivorous damselfish by Polunin and Brothers (1989) and Klump and Polunin (1989). This pattern does, however, reflect the availability of plankton prey in the Bay of Calvi (Frangoulis, 2002), since at dusk there is movement of zooplankton towards the surface from the so-called 'day-depth'; at midnight the zooplankton descends from the surface (*midnight sinking*) and subsequently there is a regrouping near the surface at dawn (*dawn-rise*), followed by a descent during the early hours of daylight. It was generally observed (J.K. Pinnegar personal observation) that very shortly after dawn, *C. chromis* streamed out of their shelters and began feeding rapidly, so much so that within 10 min of sun-up, no adult individuals could be found within shelters. In the evening, prior to dusk, *C. chromis* formed large gatherings over the substrate, but appeared to continue to feed, possibly consuming the abundant meroplanton e.g. mysids, ostracods and caprellids which rise from the substrate during this period (Fage, 1933), although this is not supported by Dulčić (2007) who found few such items in the stomachs of *C. chromis* in the eastern Adriatic. The bimodal pattern in the feeding rate of *C. chromis*, around or shortly after the crepuscular periods, may be common among planktivorous fishes and has been reported for other species (e.g. Woodhead, 1966).

Noda et al. (1992) did not find a clear bimodal feeding pattern in the closely related planktivorous damselfish *Chromis chrysurus*, although there is much evidence to suggest that planktivorous damselfish respond markedly to changes in food availability. Hobson and Chess (1976) suggested that *Chromis punctipinnis* feed more rapidly in a current than they do at slack water, and Noda et al. (1992) postulate that *C. chrysurus* may store fat in the body by gorging on densely aggregated patches of zooplankters when they occur, thereby accumulating sufficient food reserves overall. Gorging behaviour was not, however, observed by Bray (1981) or de Boer (1978), and indeed feeding rates of adult *C. chrysurus*
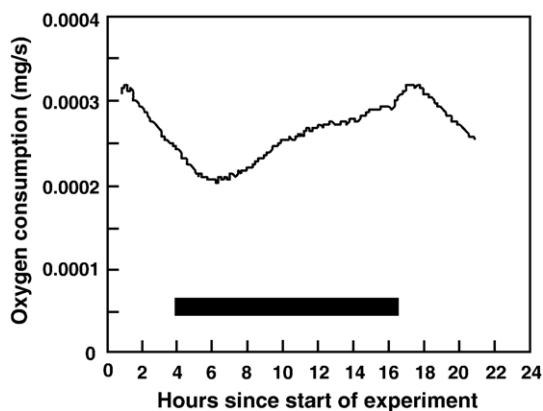


Fig. 4. Experimental record of oxygen consumption patterns over the course of 24 h in a single *Chromis chromis*, weighing 14.85 g wet mass. (Black bar indicates period of darkness).

under strong currents were as low as those under weak flows, despite migrations over considerable distances on a daily basis to the incurrent end of the home range to forage. Noda et al. (1994) described a situation in which shoals of C. chrysurus stayed at feeding sites for longer than an hour, irrespective of the presence or absence of prey.

### 4.3. Production

The relationship between fish length and weight has been studied for C. chromis at many sites (Dulčić and Kraljević, 1995; Duka and Shevenko, 1988) and there are clear differences among these studies in the growth exponents. Sexual maturity, hydrological conditions and differing food availabilities can all affect the growth exponent (Dulčić and Kraljević, 1995).

The values we obtained for the von-Bertalanffy growth function indicate that C. chromis is a relatively slow-growing fish ($k = 0.464, 0.486$) with respect to other dam-selfish species, although Dulčić and Kraljević (1995) report even slower growth for C. chromis in the central Adriatic ($k = 0.26$). Isaac (1990) suggested that both ELEFAN and SLCA tend to underestimate $k$ and over-estimate $L_\infty$, and this may explain the apparent discrepancies with the values given by Dulčić and Kraljević (1995) who used readings from scales in order to calculate growth parameters. An alternative explanation might relate to fishing pressure, since it is widely appreciated that where stocks are exploited, individuals generally grow more slowly and attain lower total lengths. Dulčić and Kraljević (1995) stated that damselfish form a small but significant component of the coastal beach-seine and gill-net catch ($\sim 30$ tons/year) in the middle Adriatic and estimate a fishing mortality ($F$) of 0.35 year$^{-1}$. Fishing pressure on C. chromis is much lower in Corsica, al-though some are still caught as by-catch by traditional beach-seines (J.K. Pinnegar personal observation). Pin-negar and Polunin (2004) estimated fishing mortality of C. chromis in the Bay of Calvi at 0.10 year$^{-1}$.

Isaac (1990) proposed that ELEFAN is more effec-tive in producing correct results for faster growing (i.e.

fish with $k > 0.5$) short-lived fish, whereas SLCA is more suited to slow-growing, long-lived fish. On this basis, SLCA may be more suited for use with C. chromis, which can apparently reach an age of 8 or 9 years.

The allocation of carbon and nitrogen to somatic tissues ($P_s$) is apparently much higher in C. chromis (Table 3) than in the herbivorous damselfish Plectro-glyphidodon lacrymatus (Polunin and Brothers, 1989). Gross growth efficiency depends on size, age, diet ration and environmental conditions. Brett and Groves (1979) concluded that gross energy conversion to growth in herbivorous fish is not likely to exceed 20% while values of up to 50% can be achieved by carnivorous juveniles. Very few data are available concerning growth efficiency in relation to nutrients such as carbon, nitrogen and phosphorus, but Pandian (1967) showed that nitrogen GE declines with increasing fish size in two carnivorous species (Megalops cyprinoides, Channa striata), and the values reported here for C. chromis are similar to Pandian's values for adult fish ($\sim 13\%$).

### 4.4. Reproduction

Polunin and Brothers (1989) suggested that the fate of their 'missing' carbon and nitrogen in P. lacrymatus could be related to high nutrient allocation to reproduc-tive tissues, since even in temperate waters, there is evidence that some species may regularly allocate 40% or more of ingested energy to gonadal tissue production (Rogers, 1988). However, our estimates for the alloca-tion of nutrients to reproductive tissues in C. chromis are actually very low (Table 3, Fig. 3). Prior to the present study, the most detailed work to have focused on C. chromis was that of Contini and Donato (1973), who provided a qualitative account of gonad maturity/activity over the course of a year. Contini and Donato (1973) suggested that, during winter, both male and female gonads go through a period of rest, and that spermato-genesis and oogenesis proceed from April to June, reaching full maturation between July and September when temperatures reach 24–26 °C. Dulčić and Kraljević (1994) showed that C. chromis fecundity (the

Table 3
Proposed carbon, nitrogen and phosphorus budgets for a 10.25 g (wet weight) individual of Chromis chromis, in the Bay of Calvi, Corsica

| Element | C | A | F | A′ | U | R | $P_s$ male | $P_s$ female | $P_r$ male | $P_r$ female |
|---|---|---|---|---|---|---|---|---|---|---|
| Carbon | 42.05 | 35.32 | 6.73 | 4.85 | 15.45 | 15.02 | 4.56 | 4.16 | 0.30 | 0.69 |
| Nitrogen | 9.69 | 9.01 | 0.68 | 0.80 | 8.21 | 0 | 0.72 | 0.66 | 0.08 | 0.14 |
| Phosphorus | 1.91 | 1.04 | 0.87 | 0.21 | 0.83 | 0 | 0.20 | 0.20 | 0.01 | 0.01 |

All values are expressed in mg/day and $C$ is consumption, $A$ is absorption, $F$ is faecal output, $A′$ is assimilation, $U$ is excretion, $R$ is metabolism (respiration), $P_s$ is somatic growth, $P_r$ is gonadal growth.
A′ is based on mean $P_s$ and mean $P_r$ values for both sexes.

number of mature eggs) increased exponentially with fish age/length and that female damselfish matured at 6.8–7.3 cm (TL) as they approached age 2. These results agree with our findings for *C. chromis*, in which ovary mass increased exponentially with fish mass, and the smallest individual with mature looking ovaries was 8.38 cm (9.48 g).

The bioenergetics of ovarian maturation have been studied in many species. Wooton et al. (1980) showed that in the three-spine stickleback *Gasterosteus aculeatus*, ovarian growth represented from 1 to 3% of total daily energy income. Diana (1983a) showed that the annual investment of energy in reproduction by female pike (*Esox lucius*) was 11–16% over the second, third and fourth years, while somatic investment declined from 42 to 5–8%. Few studies have investigated the allocation of nutrients to gamete production, and our work on *C. chromis* suggests that carbon, nitrogen and phosphorus allocation to this function is very much less than the allocation of energy. Wooton (1979) suggested that successful spawning does depend on the allocation of nutrients and minerals to developing oocytes.

Testes frequently represent a much lower proportion of body weight than ovaries (Wooton, 1998), and in *C. chromis* the mass of ovary in a 10.25 g fish would be approximately 0.76 g, but the mass of testes in fish of the same size would be only 0.61 g. Consequently, allocation of carbon and nitrogen to reproductive tissues in male fish is also much less than in female fish. However, despite their large investment in eggs, average growth of females (adjusted for size) does not seem to be slower than in males (Table 2, Dulčić and Kraljević, 1995). Other than the general observations on spermatogenesis by Contini and Donato (1973), nothing is known about the allocation of resources to reproductive function in male *C. chromis*. Here we have shown that unlike in females, the mass of mature male gonads seems to level out to an asymptote (Fig. 3b), and that testes increase from 1.55% of total body mass in 3.60 g fish to 3.79% in a 33.81 g fish. Diana (1983b) suggests that in male pike, the annual allocation of energy to reproduction increased from 0%, in the first year of life, to 4–5% in the next three years of life, but it appears that allocation of carbon, nitrogen and phosphorus to this function in *C. chromis* is very much lower. van Rooij et al. (1995) suggested that there was no significant difference in the growth parameters of male and female *Sparisoma viridae* because despite lower investment of energy in gamete production by males, males invested relatively more energy in behaviour related to reproduction, such as female acquisition and territorial defence. Wooton (1998) discussed allocation of resources

not only to gonadal production but also the costs of developing secondary sexual characters and to reproductive behaviour. Clearly energy and resources spent on reproductive behaviour are not available for activities that would contribute to somatic growth and maintenance. Male *C. chromis* exhibit complex breeding behaviour (Mapstone and Wood, 1975; Picciulin et al., 2004) involving the preparation, clearing and guarding of a nest.

## 4.5. Respiration

The quantity of oxygen consumed per gram of fish tissue per day (1.7 mg) was considerably lower than the 3.62 mg $O_2$/g/day measured for the same species by Vernon (1895). However, this very early study used less precise volumetric methods and was based on a single (10.4 g) fish, as opposed to the present study, which used continuous monitoring of five individual fish, over a range of sizes. Both values do, however, fall within the range reported for *C. chromis* by Altman and Ditmer (1971).

Three levels of metabolism are defined by fish physiologists (Brett and Groves, 1979; Wooton, 1998). 'Standard metabolism' ($R_s$) is the rate of expenditure in a resting unfed fish, i.e. approximately equivalent to the minimum rate required to keep the fish alive. 'Maximum active metabolism' ($R_{max}$ or $R_a$) is the maximum sustainable aerobic rate (the difference between $R_{max}$ and $R_s$ defining the fish's scope for activity). The 'routine' rate of metabolism ($R_{rout}$) is the metabolic rate of an unfed fish, but showing spontaneous swimming (Wooton, 1998). The measurements here are roughly equivalent to the routine rate of metabolism, although undoubtedly some of the carbon apportioned (according to mass balance) to 'excretion' (Table 3 and Fig. 5) was actually lost via metabolism associated with activity and behaviour. Indeed, Polunin and Brothers (1989) suggested that this may be one of the main outcomes for 'missing' carbon in the herbivorous damselfish *Plectroglyphidodon lacrymatus*. It has been suggested (Brett and Groves, 1979) that as much as 60% of the metabolizable energy in fast-growing carnivorous fish may be used in maintenance, activity and feeding, and Soofiani and Hawkins (1985) showed significant increases in the oxygen consumed during feeding periods, implying that the present estimate for carbon production may be a considerable underestimate.

In the present experiments, fish showed fluctuations in the oxygen consumption rate (Fig. 4) throughout the day, with peaks (indicating increased activity) in the morning, shortly after the lights were turned on. Similar
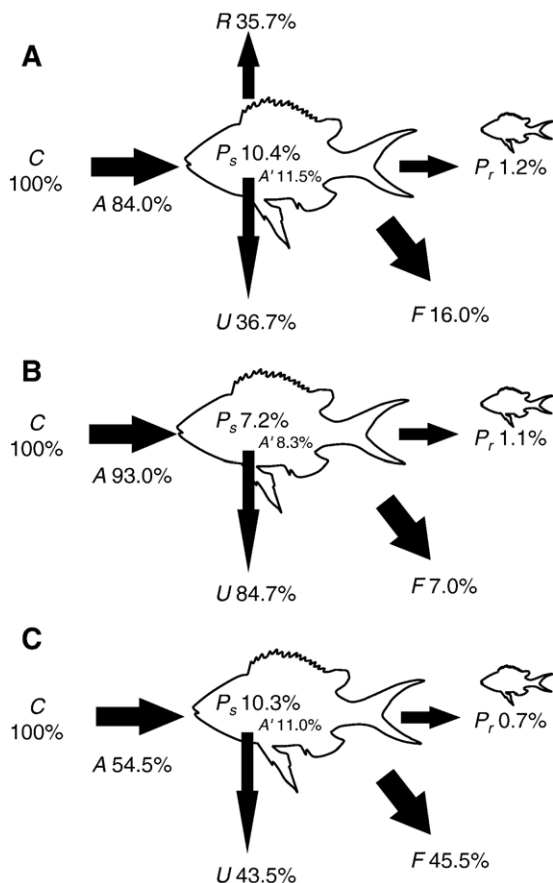
Fig. 5. Proposed carbon (A), nitrogen (B), and phosphorus (C) budgets based on a 10.25 g (wet weight) individual of *Chromis chromis*, in the Bay of Calvi, Corsica. All values are expressed as a percentage of the amount ingested and $C$ is consumption, $A$ is absorption, $F$ is faecal output, $A'$ is assimilation, $U$ is excretion, $R$ is metabolism (respiration), $P_s$ is somatic growth, $P_r$ is gonadal growth.

variability was noted for captive parrotfish (*Sparisoma viride*) by van Rooij and Videler (1996).

### 4.6. Excretion

Essentially all of the inorganic phosphate that leaves the body of a fish is excreted by the kidneys (Grafflin, 1936). In this study it was estimated that approximately 43.5% of all phosphorus ingested was excreted as dissolved phosphorus, and this agrees well the observations of Kraft (1992) for plankton-feeding young-of-year yellow perch (*Perca fluvescens*). Braband et al. (1990) found that 85–95% of the phosphorus released (by *Rutilus rutilus*) was in the form of soluble molybdate-reactive P, with the remainder precipitated as faecal pellets. This focusing of available phosphorus by planktivorous fish has been suggested to be of vital importance to the internal

recycling of phosphorus in lakes (e.g. Braband et al., 1990; Kraft, 1992), and it may also be of considerable importance in the Mediterranean where phytoplankton and most littoral macrophytes are severely phosphorus-limited (Atkinson and Smith, 1983).

It appears that 12 times more nitrogen is produced by *C. chromis* in the form of dissolved nitrogenous compounds than is egested as faeces (Fig. 5). This pattern seems to be a general characteristic of most carnivorous species (Pandian, 1967; Meyer and Schultz, 1985a,b; Leung et al., 1999), but Polunin and Koike (1987) showed that the opposite is true in the herbivorous damselfish *P. lacrymatus*. In most teleosts, ammonia is the principle end product of protein catabolism (Smith, 1929) and is the major compound of nitrogen excretion. Other excreted nitrogenous compounds include urea, creatine, uric acid, and trimethylamine oxide. Smith (1929) showed that essentially all the ammonia and urea released originates from the gills, while amine oxide and less diffuse nitrogenous products are excreted by the kidney. In the planktivore *Brevoortia tyrannus*, exogenous nitrogen-excretion represented 61.6% of all the nitrogen ingested, and the percentage of this excreted nitrogen produced in the form of ammonia was around 69.6% (Durbin and Durbin, 1981). Using data from Bray et al. (1986) for the planktivorous damselfish *C. punctipinnis*, it is possible to estimate that a fish of an average wet mass of 62.4 g, would excrete 18.06 mg of nitrogen per day or 0.3 mg/gram of tissue/day, and this is less than the quantity of nitrogen excreted in the present study, by the closely related *C. chromis* (0.8 mg/g/day). Bray et al. (1986) reported consistently-elevated concentrations of ammonium within or just downstream of *C. punctipinnis* aggregations, and Webb et al. (1975) speculated that increased ammonium concentrations around isolated coral pinnacles were due in part to resident damselfish (*Dascyllus* spp.). Furthermore, Bray et al. (1986) recorded even higher ammonium levels in crevices used by *C. punctipinnis* as night-shelters, and it was estimated that this species excretes ten times the amount of ammonia onto the reef than all other resident macro-invertebrates and fish put together (Bray et al., 1988). Ammonium is an important source of nitrogen for many primary producers in reef ecosystems (Meyer et al., 1983), and the ammonium produced by the fish *C. punctipinnis, Haemulon flavolineatum, Haemulon palmata* has been implicated in more rapid growth of macro-algae and coral polyps around sites where these species rest (Bray et al., 1986; Meyer et al., 1983; Meyer and Schultz, 1985a,b). The potential for *C. chromis,* as the most abundant littoral species in the Mediterranean, to serve a similar function must be considered seriously.

### 4.7. Balancing the budgets

One of the greatest obstacles to balancing the nutrient budgets has been the paucity of phosphorus in the plankton food that the fish consume. Assuming a daily ration of 120.2 mg (dry), and a phosphorus content of 0.68% (Table 1), then a 10.25 g fish is expected to consume approximately 0.82 mg of phosphorus each day. However, measured amounts of phosphorus excreted, defaecated and assimilated (Table 3), were more than twice the amount thought to be ingested. If phosphorus is available in such small quantities, and much of the Mediterranean has been suggested to be highly phosphorus-depleted (Berland et al., 1980; Krom et al., 1991), then it seems somewhat surprising that *C. chromis* should egest as much as 45.5% back into the environment as faeces (Fig. 4). A similar situation was suggested for *C. punctipinnis* by Geesey et al. (1984), whereby faeces were significantly enriched in phosphorus compared with undigested stomach contents. It is possible that *C. chromis* somehow supplements its normal diet with a phosphorous-rich food, but possible candidates are unclear. Bazely (1989) has suggested that many phosphorus-limited terrestrial herbivores on islands occasionally eat seabird bones or faeces to supplement their phosphorus intake. Here (Table 3), the amount of phosphorus which would theoretically be needed to support a 10.25 g fish was estimated, in accordance with the amount excreted, assimilated and defaecated (P in faeces calculated using the measured C:P ratio of faeces and the quantity of carbon egested).

In the carbon budget of *C. chromis* (Fig. 4a), as much as 36.7% of all ingested carbon was suggested to be apportioned to excretion (U). As already stated, this was treated as the 'default' in the mass balance equation, and much of this carbon may in fact be associated with carbon dioxide production during periods of activity or feeding. However, other potential outputs of excreted carbon must be considered, including the carbon in organic nitrogenous compounds such as urea, creatine, uric acid and trimethylamine oxide produced in the fish's kidneys and gills and the large quantities of carbon excreted in the form of exudates such as mucus. Mucus plays numerous roles in teleosts, including respiration, ionic/osmotic regulation, reproduction, egestion disease/pathogen resistance, communication, nest building and feeding (Shephard, 1994). On some fish surfaces, e.g. gills and skin, a significant and virtually continuous layer of mucus exists and is constantly being sloughed off and replaced. In corals, urchins and many other animals, mucus production has been purported as a means of excreting excess carbon where they have to consume large amounts of 'low quality' food in order to obtain sufficient quantities of other nutrients such as nitrogen and phosphorus (Bythell, 1988).

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