Growth and reproductive simulation of candidate shellfish species at fish cages in the Southern Mediterranean: Dynamic Energy Budget (DEB) modelling for integrated multi-trophic aquaculture

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1. Introduction

Integrated multi-trophic aquaculture (IMTA) is a practice that couples low trophic (e.g., suspension feeders) to higher trophic fed (e.g., fish) organisms so that the nutrient waste of one species can become nutritional inputs for another (e.g., shellfish; Reid et al., 2010; Troell et al., 2003, 2009). IMTA effectiveness has been demonstrated in both fresh and marine waters by coupling many different types of organisms (FAO, 2009). Shellfish growth is highly adaptable to a wide range of temperatures and diet varieties making them a highly successful global aquaculture species (Barrington et al., 2009) well suited for IMTA (Sarà et al., 2009; Troell et al., 2009). Shellfish acquire energy filtering suspended particles such as phytoplankton and detritus (Reid et al., 2010; Sarà, 2006, 2007a; Sarà et al., 2000, 2003). Gradients of organic particulates as indicated by shellfish uptake (Gao et al., 2006; Jones and Iwama, 1991), inorganic soluble nutrients (Reid et al., 2006; Sanderson et al., 2008; Yokoyama and Ishii, 2010) and phytoplankton (Sarà et al., 2007), have been reported close to fish cages, although often with extensive spatial and temporal variation (Dalsgaard and Krause-Jensen, 2006; Pitta et al., 2009; Sarà, 2007a, 2007b, 2007c; Sarà et al., 2009, 2011b). In open-water, IMTA systems, proximate shellfish can potentially benefit from solid organic particulates (faeces and waste feed) directly from the fed trophic level (i.e. fish); or through a step wise process of soluble nutrient loading (ammonium phosphate) driving an increase in primary production (PP). These two possible routes of augmented diet production for IMTA shellfish will be a function of several mechanisms, such as fish farm scale, temporal scales (time of PP response to a nutrient load, which can range from hours to days respectively), ambient nutrient concentrations, temperature and hydrodynamics (Aure et al., 2007; Troell et al., 1999, 2011).

To date most studies on IMTA effectiveness have been derived from empirical field research (e.g., Cheshuk et al., 2003; MacDonald et al., 2011; Sarà et al., 2009). However, IMTA site design and feasibility studies could benefit significantly from a modelling approach capable of mechanistic predictions for the growth of shellfish under a variety of temperatures in conjunction with varying diet concentrations. Specifically this would address important planning criteria for IMTA development by...
DEB theory is explicitly based on the conservation of mass, isotopes, energy and time, including the inherent degradation of energy associated with all processes (Kooijman, 2010). It uses surface area and volume relationships to keep track of three state variables of all organisms: i) the reserve density, ii) the structural volume and iii) maturity. Energy and matter assimilated from food are assumed to be proportional to the organisms’ surface area following Holling’s type II functional response curve (Holling, 1959) and initially directed to the reserve (e.g., fat, carbohydrate, protein) pool (Fig. 2), which is distributed throughout the tissue. The permanent biomass (e.g., proteins and membranes) requires energy for its maintenance in direct proportion to structural volume (Kearney et al., 2010). Reserve is mobilised at a rate in accordance with the weak homeostasis assumption. This flux is allocated according to the κ-rule (Kooijman, 2010): a fixed fraction (κ) is allocated to growth and somatic maintenance when the remainder fraction (1-κ) is allocated to maturity maintenance plus maturation or reproduction (Fig. 2). Life stages exploit energy in different ways: i) juveniles mature and increase their complexity (i.e., develop new organs and regulation systems), and ii) adults reproduce. Hence, the energy spent on development in juveniles will be exploited on reproduction in adults. If the general condition of the external environment in which organisms live is poor (i.e., deviated values of temperature and food availability with respect to the common natural patterns), reproduction and growth are reduced and in extreme situations, rejuvenation and shrinking might occur (Kooijman, 2010). Maintenance has priority over growth and consequently growth ceases when all mobilised reserve is required for somatic maintenance. The standard DEB model also assumes isomorphy (i.e. no change in shape during growth, like shellfish –Van der Meer, 2006).

2.2. Step 2: water temperature dataset

In DEB models, the main forcing driver of shellfish life history is represented by mean seawater temperature (Kearney et al., 2010; Kooijman, 2010; Pouvreau et al., 2006; Sarà et al., 2011a). The DEB models were run with 4-yearly hourly data (01 January 2006–31
December 2009) of seawater temperature measured about 1 m below the surface at the closest meteo-oceanographic station held in Paler-}

mo (LAT 38° 07′17.08″; LONG 13° 22′16.79″; not more than 35 km eastern–ward far from the Gulf of Castellammare). Data are available at the Italian Institute of Environmental Research (ISPRA) web page (http://www.mareografico.it/). A period of 4 years was consistent with the normal life span of most shellfish (Gosling, 1992).

2.3. Step 3: empirical measures of chlorophyll-a

Chlorophyll-a (CHL-a; as a proxy for phytoplankton biomass) is one of the more reliable food quantifiers for the calculation of DEB shellfish parameters (Pouvreau et al., 2006). Most bioenergetic experiments consider phytoplankton a reliable diet to calculate theoretical responses under varying external conditions (Egzeeta-Balic et al., 2011; Sarà et al., 2008; Widdows and Staff, 2006). As a result of chlorophyll-a measures around 2011; Sarà et al., 2009, 2011b). The fish farm was the same as the one used in Sarà et al. (2009), producing 600 tons of seabass (Dicen-}

trarchus labrax) and seabream (Sparus aurata) annually. Water samples were collected from sub surface (—1 m), —10 m and —20 m at three sites close to the fish cages (downstream within about 50 m from the cages; Sarà et al., 2006; hereafter IMP; Fig. 3) and from three sites positioned far about 1500 m western ward far from the cages (hereafter CTRL; Fig. 3). This sampling scheme was chosen in accordance with previous studies carried out in the Gulf (Modica et al., 2006; Sarà et al., 2006). Water samples were collected using Niskin bottles, samples were filtered through Whatman GF/F filters (0.45 μm nominal pore size) and analysed for chlorophyll-a (CHL-a) and saturated chlorophyll-a (CHL-a far from cages) food conditions at 12, 18, 24, 36 and 48 months from settling. ii) the potential reproductive outputs expressed as number of eggs produced per biomass unit (dry weight) and iii) the mean annual von Bertalanffy growth rate (K). We com-}

pared our model results to all available data published in the current literature on maximum size (shell length) reached in the Gulf of Cas-

pellamare waters (Sarà et al., 1998, 2009).

2.5. Statistical analysis

An analysis of variance (ANOVA) was used to test for potential dif-

ferences in chlorophyll concentrations between CTRL and IMP sites. During this analysis, condition (2 levels), season (4 levels) and depth (3 levels) were chosen as fixed factors, three sites were treated as ran-

dom and nested in condition. To test if the shell lengths and the number of eggs obtained from DEB simulations at 12, 24, 36 and 48 months were different between close and far cages at three depths, an ANOVA was applied with CTRL, IMP and three depths (0 m, —10 m and —20 m) as fixed factors. Heterogeneity of variances was tested using Cochran’s C test prior to the analysis of variance, and Student–Newman–Keuls (SNK) test enabled the appropriate means comparison. GMAV software (University of Sidney, AU) was used to run ANOVA.

3. Results

3.1. Temperature and chlorophyll-a

In the study location of the Southern Tyrrhenian, surface water tem-

peratures ranged from about 13 to 26.5 °C. In Palermo, subtidal eco-

therms could experience a body temperature (BT) normally falling inside the Mediterranean range for 83% of the time (~260 h over 4 years), a warmer BT than the upper Mediterranean limit (~26.5 °C) for about 16% of the time (~5700 h over 4 years), whilst they typically live in colder waters (~13.5 °C) than the lower Mediterranean limit (13.8–14.1 °C) for less than 1% of the time. IMP waters had
chlorophyll-a concentrations significantly higher ($p<0.05$) by about 45\% ($2.3\pm1.1$ $\mu$g l$^{-1}$) compared to CTRL waters ($1.3\pm0.6$ $\mu$g l$^{-1}$; Fig. 3). The water columns at both sites were homogeneous with no significant differences in chlorophyll-a between depths ($p>0.05$).

### 3.2. Shellfish growth

DEB simulations with local temperature and CHL-a, demonstrated that growth close to cages could reach a greater $L_\infty$ by the end of 4th year ($p<0.05$), compared to that of the CTRL (Table 2a; Figs. 4 and 5). Shellfish in this region are typically harvested at two years and growth at this time is indicated in Figs. 4 and 5. IMP mussels reached a von Bertalanffy ultimate $L_\infty$ of over 26\% compared to mussels in CTRL. No difference was detected due to depth ($p>0.05$). The von Bertalanffy growth rates, $k$, were higher in CTRL than in IMP mussels. Also, the number of eggs produced over 4 years was significantly higher in IMP (+24\%) compared to CTRL (Table 2a; Fig. 4). Maturity time of IMP mussels was faster (166\pm27 days) compared to CTRL mussels (205\pm46 days).

DEB models predicted that Japanese oysters at IMP sites reached almost 12 cm in 4 years (Table 2b; Fig. 5) and this size was significantly higher compared to CTRL oysters ($p<0.05$). This corresponded to an increment of over 35\% compared to CTRL animals. Growth constant $k$ was similar for CTRL and IMP oysters. Overall, however, no eggs were produced under the different cultivation conditions over the 4 year period.

### 4. Discussion

To date, little research on individual aspects of species fitness in conjunction with temperature and food, both at experimental or commercial scale has been investigated in an IMTA context. As aspects of our approach are novel in their application to open-water IMTA, clarification of simplifying assumptions, potential limitations, knowledge gaps and associated implications warrant further discussion (Troell and Norberg, 1998).

#### 4.1. Assumptions

The choice of primary productivity (PP) as the singular diet input for the model may seem counter intuitive in an IMTA scenario since...
organic enrichment from feed and faecal particulates are common around fish cages and these particulates have been established as a valid food source for shellfish (Lefebvre et al., 2000; Reid et al., 2000). Organic loading alone may have no immediate effect on primary production (Troll et al., 2011). Even environmental response of PP to inorganic nutrient loading from fish farms in many systems may not be detectable near cages. Increases of PP at fish cages would not be expected where ambient concentrations of limited nutrients are already very high (Rensel et al., 2007); in areas with extensive flushing such as the Bay of Fundy (Canada) where increased PP is typically undetectable at salmon cages (MacDonald et al., 2011; Martin et al., 2006), or in environments where PP response to addition of nutrients is on the order of several days (Aure et al., 2007). However, if the generation time of phytoplankton at fish cages in this study is between 0.5 and 1.5 days$^{-1}$, it is consistent with responses to nutrient enrichment in the Mediterranean, both in open waters close to fish cages (Dalsgaard and Krause-Jensen, 2006; Pitta et al., 2009), as well as during mesocosmal experiments (Duarte et al., 2000). In fact, in many fish farms worldwide, waters close to cages are significantly enriched in suspended CHL-a with respect to waters far from cages (Dalsgaard and Krause-Jensen, 2006; Pitta et al., 2009; Sarà, 2007a, 2007b, 2009).

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### Table 2a

Von Bertalanffy parameters of Mytilus galloprovincialis as estimated by DEB model at 12, 18, 24, 36 and 48 months (L$_\infty$, cm = ultimate valve length reached at the end of cultivation period; time = time in days need to reach the puberty, i.e., to first reproductive event; CTRL = far from cage area; IMP = close to cage area).

<table>
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<tr>
<th>Locality</th>
<th>Site</th>
<th>12 months</th>
<th>18 months</th>
<th>24 months</th>
<th>36 months</th>
<th>48 months</th>
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<tr>
<td>CTRL 1</td>
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<td>4.07</td>
<td>107821</td>
<td>4.51</td>
</tr>
<tr>
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<td>Surface</td>
<td>2.48</td>
<td>11.845</td>
<td>3.04</td>
<td>41800</td>
<td>3.69</td>
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<tr>
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<td>Surface</td>
<td>2.2</td>
<td>9585</td>
<td>3.15</td>
<td>20115</td>
<td>3.33</td>
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<tr>
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<td>3.63</td>
<td>62972</td>
<td>4.05</td>
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<tr>
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<td>19225</td>
<td>3.30</td>
<td>51807</td>
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<tr>
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<td>26094</td>
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<td>108486</td>
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</tr>
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<td>IMP 3</td>
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<tr>
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<td>19650</td>
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<td>64954</td>
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</tr>
</tbody>
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### Figure 4

Von Bertalanffy parameters of Mytilus galloprovincialis as estimated by DEB model ($L_\infty$, cm = ultimate valve length reached at the end of cultivation period; 4-years; $k$ rate = growth rate; $eggs$ = total amount of eggs produced over the cultivation period; CTRL = far from cage area; IMP = close to cage area). The vertical dashed line indicates typical harvest time in the Mediterranean.

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### Figure 5

Von Bertalanffy parameters of Crassostrea gigas as estimated by DEB model ($L_\infty$, cm = ultimate valve length reached at the end of cultivation period; 4-years; $k$ rate = growth rate; $eggs$ = total amount of eggs produced over the cultivation period; CTRL = far from cage area; IMP = close to cage area). The vertical dashed line indicates the typical harvest time in the Mediterranean.
2007c), in particular where hydrodynamic activity and flushing times were low.

Detailed water current measurements were not collected in our specific study area in this study. Nevertheless, increased concentrations of chlorophyll were measured down-current from fish cages. Hydrodynamic conditions favourable to localised phytoplankton growth may be facilitated from a particular morphology of the eastern part of the Gulf enabling the retention of waters near-field and downstream from fish cages (sensu Sarà et al., 2006, 2011b). However, future model development would benefit from detailed measures of surrounding current dynamics in investigation of PP response time to nutrient inputs.

4.2. Temperature considerations

To assess the feasibility of shellfish growth at fish cages in the context of IMTA, it is important to understand how temperature, in conjunction with an augmented food source (via the fed species in the IMTA system) affects growth and fitness of candidate shellfish species. For example, although M. galloprovincialis is an autochthonous Mediterranean species, it is more frequent throughout the northern coasts of the Basin (Northern Tyrrhenian, Adriatic). In the southern waters, it is patchy in distribution except for highly eutrophicated (e.g. Augusta Bay, Southern Sicily, Italy) or shallow waters (e.g. Bizzerta Lake, Tunisia). Species distribution also depends on the thermal range of its ecologic niche and the temperature hourly dataset used in this study enabled us to set the range of the candidate shellfish. This approach complements recent insights from the biophysical—bioenergetic approach (Helmuth, 1998; Keaney et al., 2010; Sarà et al., 2011a) highlighting reliable analysis of the thermal niche (i.e., the thermal ranges of metabolic functioning of marine ectotherms like shellfish or fish; Keaney et al., 2010) considering high-resolution temperature data (e.g., the tightest frequency as possible, at least on hourly basis; Helmuth, 1998; Sarà et al., 2011a). However, difficulties assessing the actual effect of temperature (i.e., using monthly or temperature wider frequencies, e.g., seasonal or yearly) on metabolism are inherent with any simulation. Here, we showed that body temperature (BT) of mussels and oysters (as ectotherms), in this aquaculture area of the Southern Tyrrhenian should fall inside the normal Western Mediterranean mean limits (14–26 °C; Margalef, 1985) for about 80% of the time. The remainder of time, shellfish BT bordered the thermal niche range of both species, in particular that of oysters. Although these species are the most common throughout aquaculture farms in the Mediterranean Sea, they are species which favour cold waters. To corroborate this as an example, we analysed the 2006–2009 temperature series of Livorno, in the Northern Tyrrhenian. Here, both mussels and oysters are normally cultivated and they are important for the local economy. In Livorno waters, subtidal ectotherms usually live within the normal Mediterranean water temperature range for over 96% of a 4-year period; the rest of time, they experience colder and warmer waters than the lower (−2% of time) and upper (−2% of time) limits respectively.

4.3. Maturation potential and environmental implications

An important aspect of using the DEB model enables estimation of the fecundity potential of cultivated organisms. This important biological aspect is often neglected in areas of applicative biology and ecological aquaculture. The presence of gametes as part of the somatic weight can have a significant impact on prices of harvested shellfish, whilst propagule release may foul fish cages and nets (sensu Sarà et al., 2007). Both aspects have potential impacts for the success of an aquaculture operation. Fecundity details are also necessary to understand the development of localised genetic diversity, and can assist in breeding and conservation management, especially when new culture species are introduced.

To verify impacts on shellfish growth, and consequently maturation under differing temperature regimes, we used Livorno temperature hourly series in the place of Palermo series, whilst including the experimental chlorophyll-a concentrations from the Gulf of Castellammare. Consequently, mussels would have reached about 10 cm over 4 years and produced over 67 million eggs (over 65% more)
than those produced under IMP conditions as estimated in the present paper. In contrast, Japanese oysters would maintain the same performance with no egg production under the same conditions; and this is consistent with the reproductive range reported in the literature (Lowe, 1974). This species spawns at water temperatures of 18.0–22.0 °C and temperature appears to be the main limiting factor for reproduction in the wild (http://www.ciesm.org/atlases/Grassostreagigas.html; sensu Frias and Segovia, 2010).

4.4. Model performance

Comparing the predictions on the basis of the DEB model with Sarà et al. (2009) growth data of mussels cultivated experimentally in the same area (no data are available for oysters), results were very similar. In that experiment, mussels coming from Adriatic hatcheries were transplanted into the Gulf at about 2.7 cm (Sarà et al., 2009). After 1 year of cultivation (2 years old), they reached approximately 6.0 cm, close to fish cages (i.e., IMP) and 5.0 cm far from cages (i.e., CTRL). The increment in mussel shell length was of about 55% and 46% in IMP and CTRL respectively. The DEB model predicted mussels starting from about 2.7 to 3.0 cm at 1 year (see Table 2a), achieve a shell length gain of about 45% and 36% in IMP and CTRL respectively; a difference of about 0.5–0.6 cm of shell length and 10% in terms of length increment per year. The model underestimation of 10% growth of mussels at both the control and IMP sites compared to empirical measures from Sarà et al. (2009), suggests equivalent external environmental influences (except for PP) at both locations. There may also be the possibility that different influences are coincidently affecting mussel growth equally at both locations, but this seems more remote. However, if we assume that the previous rationalisation is more feasible, this further suggests that settleable organic solids from the fish cages were unlikely to have significantly augmented the phytoplankton diet of IMP shellfish. Such particulates would be expected to settle-out long before reaching the 1.5 km distance of the control site, even under favourable current conditions, and consequently an equivalent diet would not be accessible at both locations. It is possible that suspended solids (SS) from fish cages could be available to IMP shellfish and these could potentially travel the distance to the control shellfish. However, the concentration of SS would be expected to be much higher closer to the fish cages, and similar concentrations would be unlikely to manifest at up-stream control sites. Unless there is evidence that SS concentrations in the Gulf of Castellammare are influenced by a homogenous mixture of SS from fish cages, it is unlikely that shellfish in either location filtered significant amounts. There is also another possibility to consider. Particulates from fish cages may be intermittently directed as shellfish as short intensive pulses of a high concentration, overwhelming filtration capacity and generating pseudo-faeces as suggested by Troell and Norberg (1998). However, in any of these scenarios it is difficult to ascertain specifically if, and how, any shellfish in the study benefited from the direct consumption of fish farm organics.

Nevertheless, consumption of detritus and fish farm organics by shellfish does occur (Bourles et al., 2009; MacDonald et al., 2011; Mazzola and Sarà, 2001; Sarà et al., 2003; Saravia et al., 2011a, 2011b) and future refinements to DEB model inputs could accommodate this. It is ultimately absorbed dietary energy in general which a driver regardless of diet type is. Consequently, knowledge of the relative consumption of fish-farm organics, their associated energy, and absorption efficiency, would also provide appropriate information to partition dietary energy within the model to account for direct organic consumption. With some modification, the DEB approach is also valid in IMTA environments where augmented growth of shellfish is due more to the direct consumption of organic particulates.

Despite the model’s slight underestimation of growth, we believe the approach sufficiently appropriate to use an IMTA planning tool, under a variety of different temperature regimes, where food augmentation is predominantly due to increases in PP. This could be very beneficial if the DEB model can be run with CHL-values coming from remote sensing such as satellite imagery, to assist with predictions at large scales (Kearney et al., 2010; Sarà et al., 2011a). Satellite data are widely available on many web sites maintained by government institutions like NOAA (http://www.nwifm.noaa.gov/Generic chloro.html) or the European Joint Research Center (http://emis.jrc.ec.europa.eu/4_1_gismap.php) for most coasts worldwide. Such data-sets can account for spatial differences between countries and/or local situations (Sarà et al., 2011b) and may assist in site-selection and monitoring of mariculture operations in marine environments (Brigolin et al., 2010).

5. Conclusion

Additional research is still required to improve our knowledge of physiological, biological and ecological aspects of many marine ectotherms with indeterminate growth amongst invertebrate (e.g. crustaceans, molluscs) and vertebrate (e.g. fish) candidates in an IMTA context. Nevertheless, modelling the growth and maturation potential of candidate shellfish species with DEBs, is a useful approach under a variety of temperature regimes, where PP is the predominant driver regardless of diet type. Consequently, we suggest predictive tools like the DEB model are strategically useful for the implication of IMTA or application to shellfish culture in general.

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