UNIVERSIDAD DE LAS PALMAS
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Pilot abalone production in Integrated Multi-trophic Aquaculture System

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Biologie

Table of content

List of figures

List of tables

Abbreviations

EF:	Effluent
DW:	Dry weight
HFLD:	High flow low density
GIA:	Grupo de Investigación en Acuicultura
GP:	Gross production
GR:	Growth rate
GSI:	Gonad Sexual Index
IMTA:	Integrated multi-trophic aquaculture
ISTAB:	International Study degree in Technical and Applied Biology
ULPGC:	Universidad de Las Palmas de Gran Canaria
UV:	Ultra violett

1. Introduction

1.1 Institutional context

As a part of the bachelor´s degree in the study program Technical and Applied Biology (ISTAB) at Hochschule Bremen, an internship was carried out as a part of Erasmus+ student exchange. The internship was performed between the 1st of February and the 30th of June 2017.

Subject of interest was the culture of the Canarian abalone (*Haliotis tuberculata coccinea* Reeve, 1846) in an Integrated Multi- Trophic Aquaculture system (IMTA). The internship was performed at the facilities of the Aquaculture Research Group (GIA) belonging to the ECOAQUA Institute from Las Palmas de Gran Canaria University (ULPGC), which is overall pursuing strategies of Blue Growth and sustainable use of natural resources in coastal areas. Thus, ECOAQUA covers research lines on aquaculture, fisheries, marine spatial planning or ecology. In particular, GIA is focused on several research lines aiming to contribute to sustainable aquaculture development (ULR3): The following research lines in particular pursued by GIA are:

1. Life cycle management: broodstock management and control of reproduction; larval rearing to improve fry quality and investigation on new fast growing species with high economic importance.
2. Genetic improvement: selective breeding programs and studies on molecular markers in aquaculture.
3. Sustainable feed production and nutritional requirements: investigation on raw material; feed formulation and nutritional requirements; products and consumer safety.
4. Aquatic animal health and welfare: studies on welfare and stress indices in fish as well as fish health diagnosis.
5. Aquariology and shellfish culture: development of local species culture technology for aquariology; cephalopod, abalone and bivalve culture.
6. Integrated Multi-Trophic Aquaculture development and aquaponics.

1.2 General scientific context

With the global oceans heavily pressured by fisheries, the living marine resources have been declining over the past (Salgado et al., 2015). Even though regulations for fisheries have been implemented the marine resources continue to decline. Aquaculture has become an option to reduce fishing pressure on the marine environment. With worldwide rising demand for fresh seafood aquaculture has become a flourishing industry. Over the last decade aquaculture production of fish and shellfish has grown an average of 7.7 % per year (Gjedrem et al., 2012). Consuming fish is seen to be healthier and more sustainable than consuming protein from cattle or pork (Skuland, 2015), nevertheless, modern aquaculture has to face several issues to become an even more sustainable food source in the future. The following subjects have to be taken into account to reach suitable sustainability production levels: feed production sustainability; habitat conservation; (Rigby et al., 2017), and disease control or environmental damages.

1.3 Integrated Multi- Trophic Aquaculture

1.3.1 General aspects

Conventional aquaculture is mainly based on monoculture farming while Integrated Multi-Trophic Aquaculture (IMTA) is a practice that refers to the incorporation of species from different trophic levels in the same system where the by-products (wastes) from one species are recycled to become inputs (fertilizers, food) for another. For instance, farming of fed species like finfish or shrimp, as top organisms of the artificial trophic chain, are combined with the culture of extractive species from lower trophic levels such as invertebrates, algae or plants to create a recirculating system in order to recover and convert the otherwise lost nutrients into harvestable biomass (Courtois de Viçose, 2015). Teleost fish are ammonotelic which means their nitrogenous waste primarily consists of ammonia which makes the fish waste a perfect fertilizer (Souza-Bastos et al., 2014). These systems have capabilities to reduce the impacts caused by animal excretion and feed leftovers from aquaculture activities (Alexander et al., 2016).

1.3.2 Mariculture IMTA

Mariculture IMTA can be subdivided in both, sea and land based systems which can be further specified by the combination of the farmed organisms. In IMTA production, finfish or shrimp can be cultured in ponds or net cages, in coastal or offshore areas in order to extract suspended organic material from the water column. Filter feeder organisms like mussels, clams or oysters are cultured next to the production units while seaweeds are cultured to remove the inorganic fraction. Organic waste material that has become part of the sediment can be consumed by invertebrate organic deposit feeders such as sea cucumbers, sea urchins or sea worms (Courtois de Viçose, 2015). Furthermore, several species such as mussels (Troell et al., 2009; Irisarri et al., 2015), macroalgae (Robertson-Andersson et al., 2008), grazing gastropod such as abalone (Viera et al., 2011; Largo et al., 2016) or detritus feeding fish (Shpigel et al., 2016), have been shown to not only increase their growth performance but also nutritious value when grown in different models of IMTA systems. The species most commonly used in IMTA as biofilters are *Ulva* sp. (Msuya et al., 2006; Naidoo et al., 2006; Robertson-Andersson et al., 2011; Ben-Ari et al., 2014) and *Gracilaria* sp. (Fei et al., 2000, 2002; Fei, 2004; Njobeni, 2005; Viera et al., 2006, 2009; Yongjian et al., 2008; Mao et al., 2009; Marquardt et al., 2010). *Ulva*'s high capacities for nutrient uptake are commonly known in aquaculture industries (Marínez-Aragón et al., 2002). However the harvestable biomass from *Ulva* species has a low market value. In contrast to *Ulva, Gracilaria* species can also be used to produce high value products such as agar-agar (Neori et al., 2004). Due to their fleshy morphology *Gracilaria* species display slower growth rates than *Ulva,* who grow in flat sheets of double layered cells (Marinho-Soriano et al., 2002; Nagler et al., 2003).

1.4 Abalone as suitable species in IMTA

1.4.1 Morphologic description, general biology and taxonomy

Abalones are marine one-shelled gastropods, also called sea ears due to their ear-shaped shell. They all belong to the family Haliotidae, genus *Haliotis* (Table 1). There are about 56

species distributed worldwide (Geiger2000). *Haliotis* are herbivorous gastropods who feed mainly on seaweeds (Nelson et al., 2002; Tanaka et al., 2003).

Table 1. Taxonomic classification of abalone species

Phylum: Mollusca Linnaeus, 1758
Class: Gastropoda Cuvier, 1797
Subclass: Prosobranchia Edwards, 1848
Order: Archaeogastropoda Thiele, 1929
Superfamily: Pleurotomarioidea Swainson, 1840
Family: Haliotidae Rafinesque, 1815
Genus: Haliotis Linnaeus, 1758

The abalone shell has a row of respiration, reproduction and excretion holes on its upper shell side. These holes are called tremata which display a defining characteristic of the abalone (Mgaya, 1995; SEAFDEC Aquaculture Department, 2000). Abalones are gonochoristic, (Stephenson, 1924), mature males and females can easily be recognized by the differences in gonad color (Bardach et al., 1972). Male individuals have cream-white gonads whereas female gonads are colored dark grey to violet (Crofts, 1929). Abalones prefer shallow sublittoral rocky habitats which are supplied by a frequently drift of algae carried by currents whereby the quantity of these drifts affects the animal density (Clavier & Chardy, 1989). The optimum habitat has fissures and crevices in its bedrock to provide shelter from predators and waves (Clavier & Chardy, 1989). They start to feed immediately on algae biofilm after larval settlement (Tutschulte and Connell, 1988). As they grow, they begin feeding on macroalgae and in the wild may change from one species to another as they mature .The color of the shell can vary from grey to red depending on the surrounding rock substrate and the algae diet (Crofts, 1929).The abalone distributed in the Canary Islands *H. tuberculata coccinea* is a subspecies of the European abalone *H. tuberculata* (Linneaus 1758). Its habitat is reaches from the intertidal zone into a depth of 15 m in semi-exposed and exposed areas. They feed on assemblages of diverse drifting macroalgae and grow maximum shell size averages about 8 cm in shell length (Espino and Herrera, 2002; Viera, 2014)

1.4.2 Abalone market situation and development of culture production

With a price of 69 € per kilogram (Legg et al., 2012) abalones are considered a product of high value. Traditionally consumed in Asia (mainly China and Japan) a rising demand for abalone has recently appeared in countries such as France, USA, Mexico, New Zealand, South Africa and Australia as well (Schiel, 1992; Guzmán Del Proó, 1992; Mcbride & Conte, 1996; Ponce-Díaz et al., 2000; Freeman 2001, Smith, 2011). While the flesh is used for culinary proposes, the shell has been used for jewellery and decoration. However the observed decreased of natural stocks worldwide are insufficient to supply the increasing demand. The high value of abalone flesh and the increasing demand, have led to an interest in mariculture of abalone species since the late 1970s (Mgaya, 1995; Viera 2014). The worldwide fishery production of

abalone declined from 20.000 tons in the 1970s to about 6.500 tons in 2015. In contrast, abalone production from aquaculture has exponentially to reach 129.287 tons in the same period of time (Cook, 2016).

In general abalone growth is seen to be slow and variable (Sales and Britz, 2001). It is affected by culture parameters such as initial size, stocking density, water renewal as well as physical--chemical parameters like temperature illumination and water quality (Viera, 2014). Another key factor for the successful culture of abalone species is the availability of suitable feed (Viana et al., 2007). Abalone post larvae are fed with a mixture of the benthic diatoms such as *Amphora* sp., *Navicula incerta*, *Nitzschia* sp. and *Proschkinia* sp. (Courtois de Viçose et al., 2012), whereas the adult animals are fed with seaweeds such as *Macrocistys pyrifera* (Badillo et al., 2007), *Ecklonia maxima* (Naidoo et al., 2006), *Palmaria* spp. (Demetropoulos and Langdon, 2004), *Ulva* spp. (Boarder and Shpigel, 2001) or *Gracilaria* spp. (Reyes and Fermin, 2003).

1.5 Internship objective

Since the awareness of sustainable use of resources in human society has developed over the years, methods like IMTA have evolved in order to reduce the anthropogenic impacts on the environment. IMTA has become a promising solution to reduce these impacts. Hence IMTA is increasing food and energy efficiency and therefore its sustainability has also enhanced the profitability of modern aquaculture. Although IMTA has not been applied to the majority of production sites, future achievements will enhance its integration into economy. The objective of increasing profit will animate the industrial sector to implement IMTA models. Simultaneously interests in ecology and sustainability will be accomplished. Therefore the main internship objective is to observe and analyze the abalone production in an Integrated Multi-Trophic Aquaculture system containing all major aspects such as broodstock monitoring and maintenance, abalone hatchery and nursery activities as well as on-growing based on biofilter produced seaweeds.

2. Material and Methods

2.1 Installation description

The facilities are located in Melenara, Telde in the province of Las Palmas on the east coast of the island of Gran Canaria 27°59'32.4"N and 15°22'08.8"W) (Fig. 1-A & B).

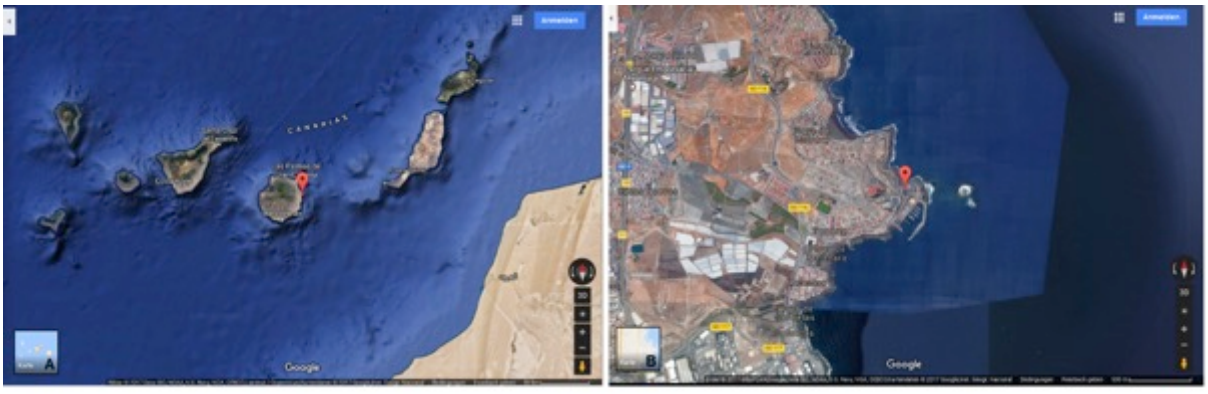

Figure 1. Location of the Ecoaqua facilities on the Canary Islands (URL4).

The IMTA model which is used by the GIA is a fish-seaweed-abalone land based system. Gilt-head seabream were co- cultured with the European abalone (*Haliotis tuberculate coccinea*) and the seaweeds *Ulva rigida* and *Hydropuntia cornea*. The finfish tanks were supplied by clean seawater and the effluents were passed through a sedimentation tank before being piped to the outdoor seaweed biofilter units. After the process of biofiltration, the water was flushed back into the sea. The system consisted of finfish rearing tanks (*Sparus aurata, Agyrosomus regius*) (Fig 2-A), abalone culture section with broodstock conditioning (Fig. 2-B), grow out area (Fig. 2-C) and abalone nursery (Fig. 2-D). Auxilary culture consisted in diatom production (Fig 2-E) and seaweed biofilters (*Ulva rigida* J.Agardh*, Hydropuntia corneaI* J. Agardh) (Fig 2-F).

Figure 2. Finfish culture tanks (A); broodstock culture site (B); grow out area (C); post larvae nursery (D); diatom production site (E); macroalgae biofilter ponds (F) (Photos: Jesse Theilen 11.05.2017).

2.2 Broodstock conditioning

The broodstock animals were kept in baskets inside 15 placed inside 60 L tanks provided with covers to reduce light penetration. The animals were kept sex-segregated. The maturity stage of the specimens was determined according to Gonad Sexual Index (Fig 3-A & B) by Ebert & Houk (1984):

0 - Immature stage, the sex is indeterminate.

1 - The gamete development has initiated; the color of the gametes is differentiated; male sex determination at this stage expressed by the creamish color; female sex determination is difficult.

2 - Gametes are well developed; sex can easily be determined but gonads are not bulky.

3 - Similar conditions like stage 2, except that the gonads are bulky. The bulk extends to the gonad tip.

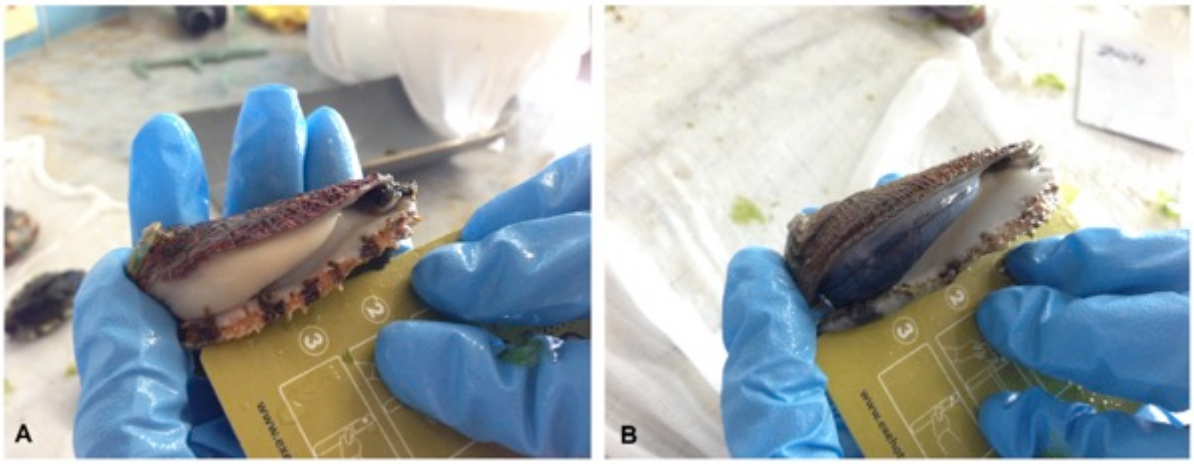

Figure 3. Male (A) and female (B) *H. tuberculate coccinea* broodstock animals with a GSI stage of 3 (Photo: Jesse Theilen 10.02.2017).

2.3 Grow out area

The grow out area consisted of seven rectangular 200 L tanks (Tarima 1-7, Fig. 2-C), two circular 500 L tanks and three circular 2000 L tanks. All animals were kept under natural photoperiod in hanged baskets within the tanks to separate the animals from the waste material. All culture units were provided with PVC shelters to offer the animals light protection and more surface area. Animals were fed with both macroalgae produced in biofiltering units and in case of shortage they were fed with artificial diets.

In order to determine the growth of the abalone, the shell growth rate (SG) and weight gain (WG) were calculated according to the following formulae.

$$\boldsymbol{SG = \frac{(SL_2 - SL_1)}{t}\ x\ 1000}$$

$$\boldsymbol{WG\ (\%) = \frac{(w_2 - w_1)}{w_1}\ x\ 100}$$

The shell growth rate is expressed in µm d^{-1}, where SL_1 displays the initial shell length mean, SL_2 the final shell length mean at the time t (days of culture). Referring to the relation between biomass increase and initial weight the weight gain was calculated and displayed in percentage, where W_1 displays the initial weight mean and W_2 the final weight mean.

2.4 Hatchery and nursery area

Males and females mature broodstock were induced to spawn by the UV spawning method (Kikuchi and Uki, 1974) or the hydrogen peroxide one by Morse et al. (1977). The gametes were fertilized during 30 min before being transferred to the larval tanks. Once hatched out, the larvae were reared in 120L tanks, maintained with flow through conditions during approximately 72h in 1 micron filtered and UV sterilized seawater. In the following stage the larvae were transferred to nursery area that consist in four rectangular 2500 L raceways which are equipped with vertically disposed polycarbonate settlement plates. The water inflow was three stage filtered seawater (25 µm, 10 µm and 1 µm). All four post larvae tanks were provided with 12 frames which stocked 20 settlement plates each (Figure 4). During this stage the post-larvae were fed with a mixed diatom diet.

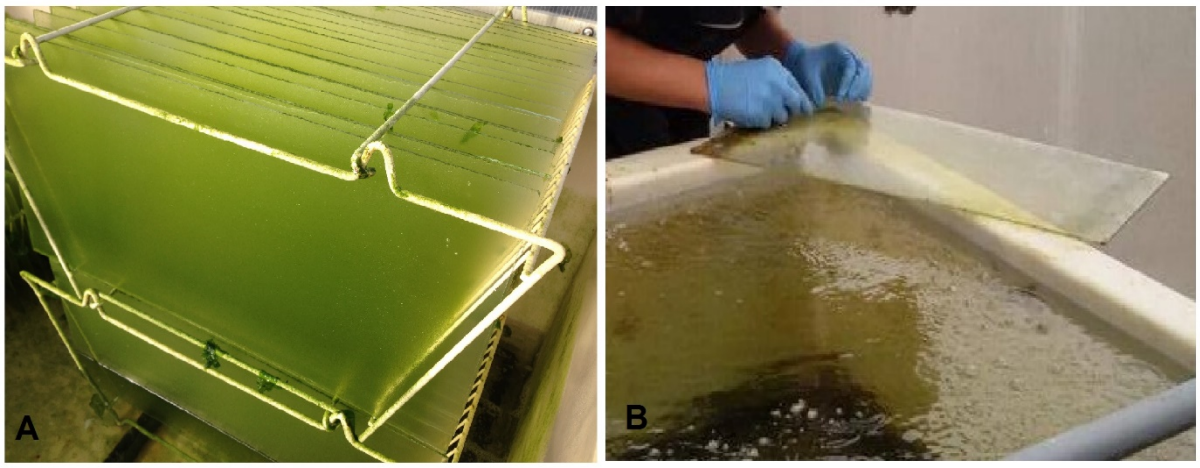

Figure 4. Settlement plates with *Ulva rigida* and *Uvella lens* cue (A) and settlement plate with diatom mixture cue (B). (Photos: Jesse Theilen 30.04.2017; María del Pino Viera Toledo 07.07.2017).

2.5 Diatoms production area

Three diatoms species (*Navicula incerta*, *Amphora* sp. and *Nitzschia* sp.) were cultured separately in 1 µm filtered, UV-sterilized and enriched with f/2-silicate medium (Fig. 5).

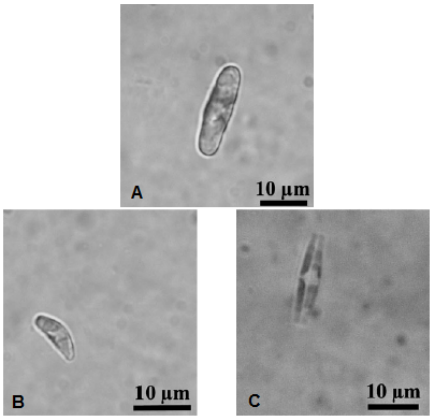

Figure 5. Benthic diatoms with lenght and width in micrometer A: *N. incerta*—18.01±0.95, 4.07±0.36; B: *Amphora* sp.—10.57±0.20, 3.67±0.11, C: *Nitzschia* sp.—18.23±0.55, 3.00±0.20 (Courtois de Viçose et al., 2012c).

The diatom culture was performed under permanent artificial light and without aeration. The diatoms strains were kept in 15 mL test tubes that were inoculated fortnightly. The mass culture of diatoms was performed by batch culture up scaling from 250 mL to 40 L.

The diatom growth was determined by using a Neubauer's hemocytometer. Before adding the suspensions to the hemocytometer, the tubes were passed through an ultrasound bath for 5 minutes to separate cell aggregations.

$$\frac{\boldsymbol{Concentration\ cells}}{\boldsymbol{mL}} = \frac{\boldsymbol{M_{5c}}}{\boldsymbol{0,004}} \times \boldsymbol{1000} = \frac{\boldsymbol{M_{5c}}}{\boldsymbol{4}} \times \boldsymbol{10^6}$$

$$Volume\ chamber = 0{,}2\ mm \times 0{,}2\ mm \times 0{,}1\ mm = 0{,}004\ mm^3 = 0{,}004\ \mu L$$

$$M_{5c}(means\ from\ 5\ counts) = \frac{C_1 + C_2 + C_3 + C_4 + C_5}{5}$$

The required amount of inoculum was calculated by the following equation:

$$\boldsymbol{D_i \times V_i = D_f \times V_f}$$

Where:

D_i = Density of mother culture

V_i = Volume needed from mother culture

D_f = Algal density at day 0 (100 000 cells/mL)

V_f = Volume total in the test tube (10 mL)

During exponential phase the growth rate μ was calculated by the formula:

$$\mu = \boldsymbol{Ln}\left(\frac{\frac{\boldsymbol{F_1}}{\boldsymbol{F_0}}}{\boldsymbol{t_1 - t_0}}\right)$$

Where:

F_1 = cell density sampled at the time, t_1

F_0 = cell density at time zero, t_0

t_1 = termination time or sample time

t_0 = initial time

2.6. Algal culture

Before entering in the seaweed tanks, fishponds effluents were channeled through the 11 m^3 sedimentation pond in order to remove suspended components from the effluent water. The macroalgae were cultured in nine 2500 L outdoor tanks under natural photoperiod. Six out of

nine tanks were under trial. One half of the tanks were stocked with *Ulva rigida* whereas the other half was stocked with the red algae *Hydropuntia cornea.* Algal stocking densities were adjusted to the optimal values obtained from previous experiments (2 and 8 g L^{-1} for *U. rigida* and *H. cornea,* respectively). Water exchange rate in the seaweed culture tanks was 12 volumes per day. Seaweeds are weekly harvested by electronic harvesting mechanisms, blotted dry and accurately weighed.

Both algal growth rate (GR) and production (GP) were weekly assessed by the following formulae according to DeBoer and Ryther (1977) respectively:

$$\mathrm{GR} = 100\frac{\ln\left(\frac{N_t}{N_0}\right)}{t} \text{ expressed in \%.d}^{-1}$$

N_t is the algae wet weight at the time *t* of the harvest, N_0 is the algae initial wet weight and *t*, is the days of culture.

$$\mathrm{GP} = \frac{\frac{\mathrm{N_t}-\mathrm{N_0}}{\mathrm{t}}\times\frac{\mathrm{DW}}{\mathrm{WW}}}{\mathrm{S}} \text{ expressed in gDW.m}^{-2}\text{.d}^{-1}$$

$\frac{DW}{WW}$ is the coefficient used to convert the algal wet weight in algal dry weight: 0.12 for *H.cornea* and 0.21 for *U.rigida*. *S* is the cultivation surface, expressed in squared meters 2.2m² for the 2500L tank (EF). Overproduction was taken to indoor storage tanks and used throughout the week for abalone feeding.

2.7 Biochemical analysis

Sampling

The macroalgae samples were taken during weekly harvest. Therefore around 500 g of fresh algae from each biofilter unit was rinsed with distilled water, blotted dry and frozen at -80 °C for later analysis (Figure 6-A). Before biochemical analysis, the algae is lyophilized (Figure 6-B). Lyophilized algae were ground to powder with a hand-held blender (Figure 6-C). The algae powder was then stored at -20 °C until analysis.

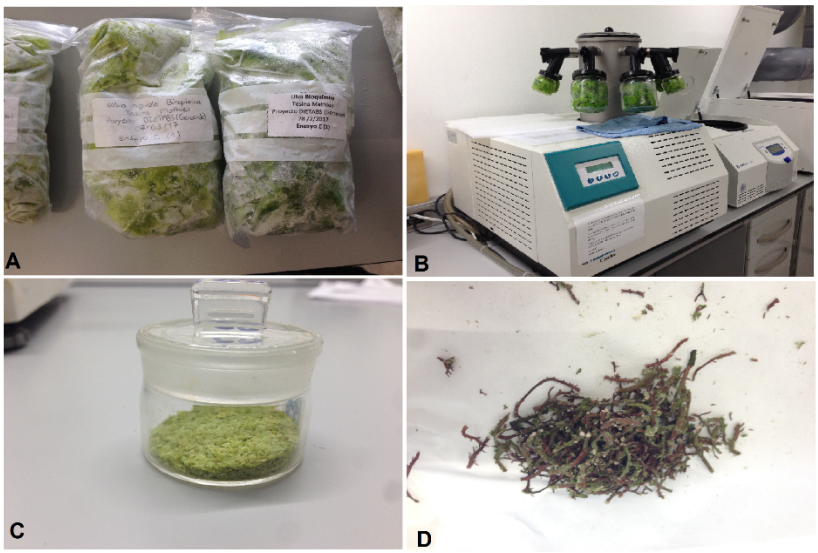

Figure 6. Freezed algae samples (A), lyophilizator, equiped with algae samples (B), grinded algae powder from *Ulva rigida* (C), lyophilized *Hydropuntia cornea* (D). (Photos: Jesse Theilen).

Ash content

Small ceramic bowls were kept in muffle furnace for 1 hour at 600 °C. Afterwards the bowls were cooled down in a desiccator for 1 hour and weight with a precision scale (accuracy: 0.0001 g) (weight A). The algae samples between 0.5 and 2 g (1 g optimum) were added with a spatula to the bowls and weighed (weight B). The samples were burned in the muffle furnace overnight at 600 °C. After the night the samples were cooled down in the desiccator for one hour and weighted again (weight C). The percentual ash content was then calculated with the following equation:

$$\boldsymbol{Ash}\ (\%) = \mathbf{100} \times \frac{\boldsymbol{C} - \boldsymbol{A}}{\boldsymbol{B} - \boldsymbol{A}}$$

Protein content

The protein content was calculated by determination of the protein nitrogen using Kjeldahl´s method (AOAC, 1995). In this method the samples with a weight between 0.2 and 0.4 g were digested at 400 °C in digestion tubes, filled with 10 mL sulphuric acid (H_2SO_4) and a Kjeldahl-copper-catalyst tab (CuSe) each, for one hour.

$$\text{Organic } N_2 + H_2SO_4 \rightarrow CO_2 + H_2O + (NH_4)_2SO_4$$

After digestion, the tubes were left under the fume hood for 25 minutes to cool off. Then 20 mL distilled water was added and mixed with a vortex. Erlenmeyer flasks were provided with 10 drops of indicator solution and 30 mL saturated boric acid. To the samples, 50 mL of NaOH 40 % was added to neutralize the excess H_2SO_4 and convert NH_4 into NH_3 which was later transferred by distillation (Mod. Foss Tecator, 1002, Höganäs, Sweden).

$$(NH_4)_2SO_4 + NaOH + Q \rightarrow NH_3 + HBO_2 \leftrightarrow NH_4^+ + BO_2$$

The distillation product mixed with boric acid and the indicator solution was titrated with HCl 0.1 N and an electronical burette. It was titrated until the color change from blue to light rose (pH 4.65).

$$BO_2 + H^+ \leftrightarrow HBO_2$$

The protein content then was calculated by the following equation:

$$\boldsymbol{Protein}\ (\%) = \frac{(\boldsymbol{V_{sample}} - \boldsymbol{V_{blanc}}) \times \boldsymbol{N} \times \boldsymbol{m} \times \boldsymbol{f}}{\boldsymbol{W}} \times \mathbf{100}$$

Where:

V_{sample} = Volume HCl for the sample [mL]
V_{blanc} = Volume HCl for the blank [mL]
N = Normality of the acid titrant
m = Nitrogen molecular weight (14.007)
f = Factor to covert the percent nitrogen in a sample to percent protein, with a value of 6.25
W = Weight of the sample [mg]

Total lipid content

The method used for the extraction of total lipids is described by Folch et al., (1957), using a mixture of chloroform-methanol (2: 1 v/v) containing 0.01 % BHT. Samples between 50 and 200 mg were mixed with 5 mL chloroform-methanol and homogenized with an Ultra Turrax (IKA-Werke, T25 BASIC, Staufen, Germany) for approximately 5 min. The Turrax was cleaned with 5 mL chloroform-methanol (2: 1 v/v, 0.01 % BHT) and 2 mL of KCl 0.88 % were added to the samples. The samples are centrifuged at 2000 rpm to separate in two phases -upper phase: salt + methanol; lower phase: lipids + chloroform. The lipids were separated from the residues by using glass Pasteur pipettes and filtering paper. The filtering paper was rinsed with chloroform to transfer remaining lipids from the paper into to test tube. From the liquid mixture, the chloroform was evaporated by N_2 in order to weight the remaining lipids with a precision scale. Afterward the percentual amount of lipids was calculated as followed:

$$\boldsymbol{Lipids}\ (\%) = \frac{\boldsymbol{Lipids}\ [\boldsymbol{g}]}{\boldsymbol{Sample}\ [\boldsymbol{g}]} \times \mathbf{100}$$

Carbohydrates

The carbohydrate content was simply defined by the resulting rest content using following formula:

$$\boldsymbol{Carbohydrates}\ (\%) = \mathbf{100} - (\%\ \boldsymbol{Ash} + \%\ \boldsymbol{Protein} + \%\ \boldsymbol{Lipids})$$

3. Results

3.1 Abalone broodstock maintenance and broodstock monitoring

On a weekly period, the abalone tanks were cleaned. Therefore the tanks were emptied and the animals were taken out and the tanks were scrubbed with chlorine and later rinsed with fresh seawater. Every morning the tanks were checked for any spontaneous spawning and possible flow rate issues. In case of a spontaneous spawning, water renewal had to be raised in order to remove sperm or eggs from the tank, to maintain a suitable water quality. While sperm could be simply removed by a higher water exchange, a female spawning involved emptying the tank and rinsing the cages and the container with fresh seawater to remove the more adhesive eggs from the cages. The animals were provided with fresh algae from the biofilters twice a week. Before feeding, the algae were left in freshwater during 15 minutes in order to remove possible parasites attached to them to reduce possible parasitic infestation that can affect shell aspects, reduction of meat quality and even lead to death. Mortality was monitored at each revision of the tanks and documented in order to determine the livestock loss.

The broodstock was monitored by measuring the shell lengths, the weight and the gonadosomatic index (GSI). The shell length was sampled with a sliding caliper with an accuracy of 0.1 mm but the sampling was rounded on half millimeter scale. The weight was sampled with a scale (Kern 440-35A, Max = 600 g; d = 0.01 g) and the gonad index was determined by visual examination and objective judgment. Means and standard derivations were calculated for each tank. The number of broodstock animals has declined from 321 to 294 with a total loss of 27 in a period of 5 month (Tab. 2).

Table 2. Total broodstock numbers and livestock loss during the training period.

Tank number	February	March	May	June	Animal loss
Repro 5	5	4	4	4	1
Repro 7	12	12	11	11	1
Repro 16	44	44	41	43	1
Repro 11	30	31	30	30	0
Repro 1	18	18	17	16	2
Repro 13	18	18	18	18	0
Repro 14	3	3	3	1	2
Repro 12	24	23	21	19	5
Repro 8	12	11	11	10	2
Repro 17	28	28	28	28	0
Repro 3	23	23	23	22	1
Repro 2	30	30	27	27	3
Repro 10	27	23	24	23	4
Repro 15	23	23	21	20	3
Repro18	24	24	24	22	2
Σ	321	315	303	294	27

Over time average shell size has increased for every reproductive tank (Fig. 7). Lowest increase in shell size is displayed by Repro 17 with an increase of average shell length of 1.1 mm (growth rate 8.5 µm/d) followed by Repro 8 (1.8 mm; growth rate 13.3 µm/d) and Repro 7 (2 mm; growth rate 15 µm/d). Repro 14 is showing a difference in average shell size of 13.7 mm between February and June. In Repro 14 only three animals were kept from which two died within the 5 month period (Tab. 2). With only the biggest animal in Repro 14 it leaves all results for this tank distorted. Therefore Repro 14 is not further considered in the analysis. Animals from Repro 1 have shown the biggest increase in average shell length with approximately 4.3 mm (growth rate 48.5 µm/d) in the time span between February and June.

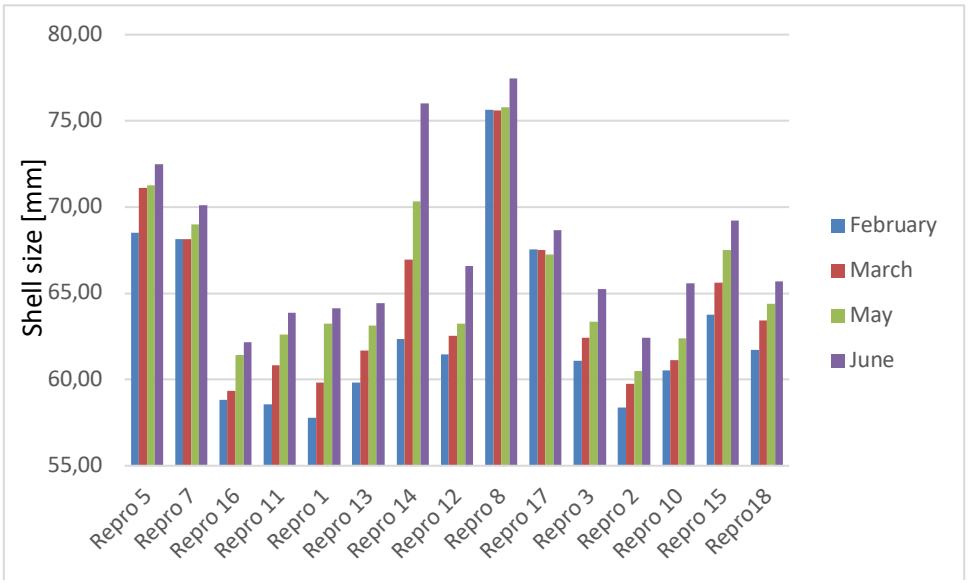

Figure 7. Average shell size of *H. tuberculata coccinea* for each broodstock tank from February to June 2017.

Repro 17, Repro 8 and Repro 7 show shell growth rates beneath 20 µm/d while the majority accomplished growth rates between 25.58 and 48.5 µm/d (Fig. 8).

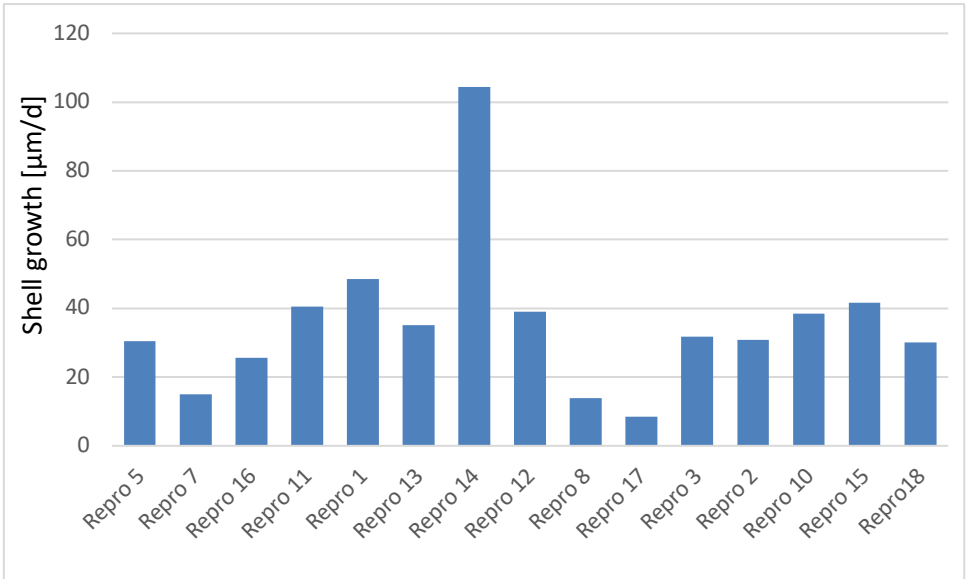

Figure 8. Shell growth rates after 5 month of culture (*H. tuberculata coccinea)* for each broodstock tank displayed in µm/d.

Two samples of same tagged 30 animals with the same age (Female from Tarima 1; Males from cestas verdes) were measured in February and June. Their growth rates and weight gain were determined. The females pictured a shell growth rate about 36.8 µm/d and the males a shell growth rate of 35.2 µm/d. Also three tagged broodstock animals (E40, E122 und E200) were used to display a shell growth rate on specific individuals. The growth rate in these three animals was 35.6 µm/d. The tanks Repro 5, 7, 14, 8 and 10 (Fig. 9) accounted a decrease of biomass due to dead and removed animals. The biggest biomass loss occurred in Repro 8 with a loss of 165.9 g (Fig. 7). The total biomass has been increased from 12.6 kg to 13.1 kg resulting in a total weight gain of 3.9 % and the average weight has increased in almost all tanks (except Repro 8) (Fig. 10). On the other hand the biggest production was performed by Repro 11 with a biomass increase of 187.9 g.

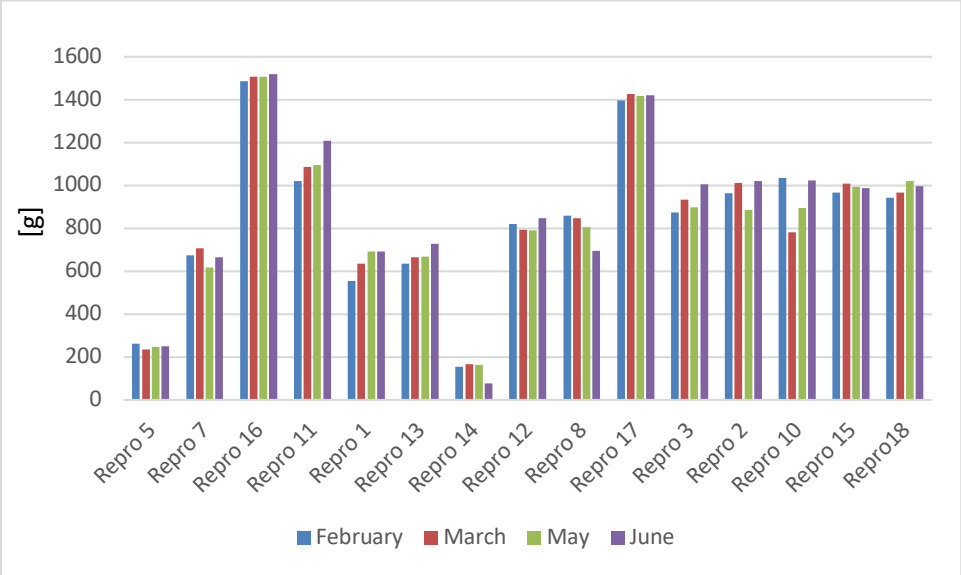

Figure 9. Biomass (*H. tuberculata coccinea*) recorded in each broodstock tank from February until June 2017.

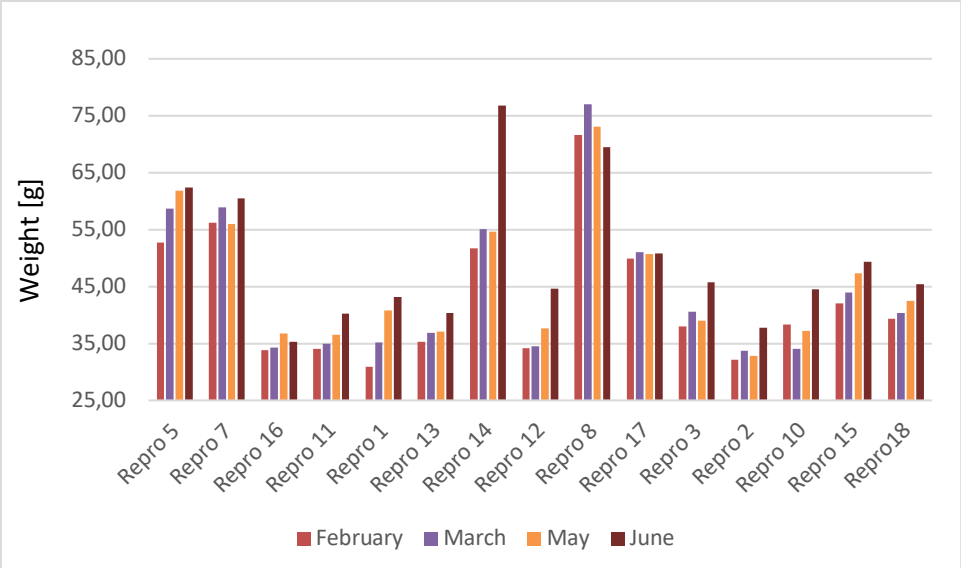

Figure 10. Average weight *H. tuberculata coccinea* for each broodstock tank from February until June 2017.

But Repro 8 showed also a decrease in average weight gain, meaning that existing animal also lost weight. There Repro 8`s weight gain had a negative value of -3.08 % (Fig. 11).

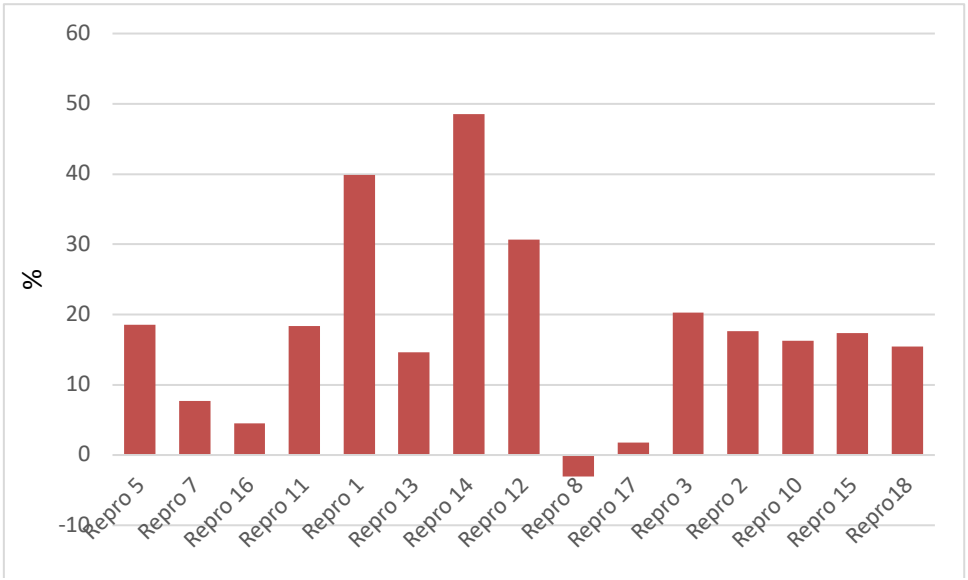

Figure 11. Average weight gain in % for each broodstock tank after 5 month of culture.

Weight gain has also been examined for the selected three broodstock animals (E40, E122 and E200) and for the sample of 30 tagged animals. The resulting weight gain for the 5 month period was 10.3 %. The sample of tagged animals shown a weight gain of 20 % for the females and 18.5 % for the males.

3.2 Abalone hatchery and nursery activities

3.2.1 Preparations, spawning and hatchery

The settlement phase is the most critical stage in abalone culture therefore before inducing the spawning the post larvae settlement tanks had to be prepared by inoculating them with spores of the macroalgae *Ulvella lens* and *Ulva rigida.*This procedure allowed the development of algal cue of *U. lens* and *U. rigida* on the settlement plates to contribute to enhance the settlement rates of the larvae introduced in the settlement tanks. During the sporulation process, the tanks were left without water input to ensure algal attachment. The sporulation was induced by an increase in the nutrient levels in the tanks. Therefore concentrated f/2 medium (easyalgae Nutriphyt; solution A 500 mL and solution B 125 mL per each tank) was added to the settlement tanks.

The spawning was induced on the 27.03.2017 and 13 male and 34 female animals with a size between 50 and 60 mm and with a GSI between 2 and 3 were selected. The animals were kept in 1 µm filtered seawater, the females were distributed onto three aerated plastic containers filled with 15 L and the males were kept in 10 L. To induce the spawning, the hydrogen peroxide method by Morse et al. (1977) was applied. Before adding the hydrogen peroxide, tris was

added to raise the pH levels to approximately 9.2 to function as a buffer. H_2O_2 was added 15 min later in proportions of 4ml/l. After adding the hydrogen peroxide the containers were covered to prevent stress from light penetration. In order to get the gametes synchronously, the females were exposed to the chemicals 30 min earlier than the males (females: 11:05 UTC tris, 11:25 UTC H_2O_2; males: 11:35 UTC tris, 11:55 UTC H_2O_2). When the animals started to release eggs and sperm, the water which was used for initiate the spawning had to be exchanged in order to ensure the viability of eggs and sperm. The eggs were siphoned from the spawning containers, filtered through a 300 µm mesh to retain waste material and passed into 10 L containers where they were fertilized with the sperm for 30 min. A total of around 1.5 Million eggs were obtained and fertilized with a fertilization rate of around 80%. The fertilized eggs were transferred to the larval rearing tanks (Fig. 12-A) after rinsing with fresh seawater in order to remove remaining excess sperm. In total three fertilizations were carried out (15:00 UTC, 16:00 UTC and 16:45 UTC). Before entering the larval rearing tanks, the larvae had to pass a separation unit (Fig- 12-B) in order to select the most vital offspring. The separation unit was provided with very little water flow. Therefore only the healthy larvae were able to swim to the upper area of the water column and were transferred through the water flow into six larval rearing tanks below.

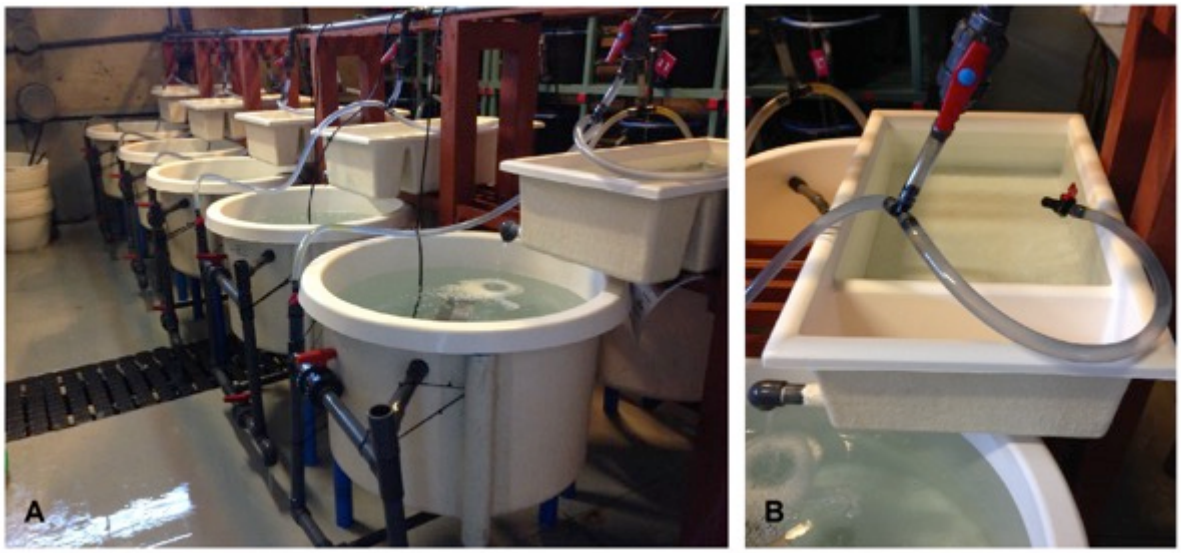

Figure 12. Larvae rearing tanks (A) and larvae selection units (B) (Photos: Jesse Theilen 28.03.2017).

After approximately 70 hours the larvae were spot checked for their development stage and competence for transfer to the settlement tanks. Therefore larvae were siphoned with a hose-pipe and passed through a 200 µm mesh. Afterward they were transferred into 10 L buckets and later to the settlement tanks. The larvae from each fertilization were transferred to their own settlement tank (15:00 UTC to settlement tank 1; 16:00 UTC to settlement tank 2; 16:45 UTC to settlement tank 3). To allow the larvae to settle the settlement tanks were maintained without water inflow for 2 days. The settlement success was estimated by visual examination of the plates in a spot tests 14 days after fertilization.

3.2.2 Diatom production

Diatom production activities started two weeks before spawning. Growth curves were compiled for the diatom species *Amphora sp.* and *Navicula incerta* from test tube cultures (Fig. 13).

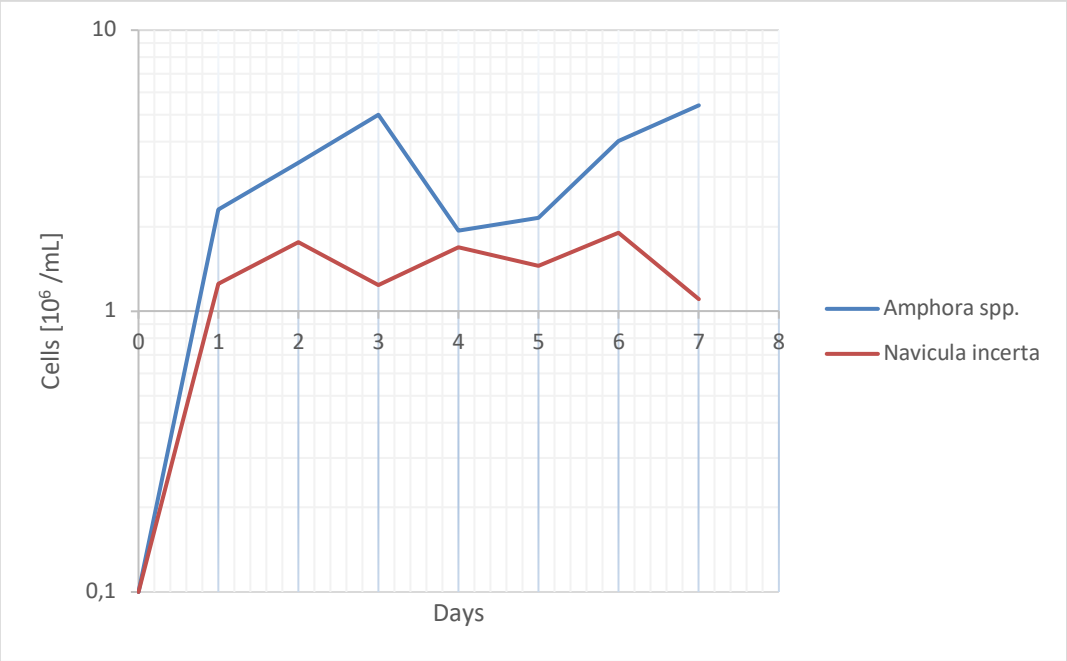

Figure 13. Growth curves of the diatom species *Amphora sp.* and *Navicula incerta* from test tube cultures.

$$\mu(\boldsymbol{Amph.}) = \boldsymbol{Ln}\left(\frac{\frac{\boldsymbol{2,3 * 10^6 cells}}{\boldsymbol{0,1 * 10^6\ cells}}}{\boldsymbol{1\ d - 0\ d}}\right) = \boldsymbol{3,14\ d^{-1}}$$

During the exponential phase (one day) the amount of *Amphora spp.* cells rose from 0.1 *10^6 cells to 2.3 *10^6 cells.

$$\mu(\boldsymbol{Nav.}) = \boldsymbol{Ln}\left(\frac{\frac{\boldsymbol{1,25 * 10^6\ cells}}{\boldsymbol{0,1 * 10^6\ cells}}}{\boldsymbol{1\ d - 0\ d}}\right) = \boldsymbol{2,52\ d^{-1}}$$

The exponential phase of *N. incerta* endured also one day, where the cell amount rose from 0.1 *10^6 cells to 1.25 *10^6 cells.

During the upscaling process, the walls of the test tubes were scraped with Pasteur pipettes to extract the most adhesive cells. The one mL volume extracted was pipetted from the test tube culture and transferred into 250 mL bottles which were filled with prepared seawater. Culturing vessels (250 mL bottles and 4 L glass balloons) were autoclaved with the filtered and sterilized seawater and afterwards enriched with the nutrient solution before being inoculated. The cells were grown for one week until they were transferred to 4 L glass balloons. In the next stage, the diatoms were cultured in 40 L plastic bags. Therefore the 4 L balloons were

shaken to homogenize the cells with the medium before pouring approximately 3.5 L of the balloons' volume into the bags for inoculation. During running progress the remaining 500 mL of the ballons' volume was used to inoculate the next charge of balloons with 250 mL of culture. The bags were harvested after a week of growing. By siphoning most of the water column with a hose the diatoms which have been growing on the bottom of the bags were separated. In the final steps, the bags were concussed in order to detach the cells from the bags and the suspension was poured into the post larvae tanks to feed the animals

After gathering all abalone spats in one tank (5.06.2017) the demand for diatom supply was lower and therefore instead of feeding the animals from 40 L diatoms bags there were fed directly from 6 L glass balloons.

3.2.3 Post larvae nursery

The settlement tanks were controlled every morning during which inflow and outflow filters were checked and cleaned. The post-larvae were weekly fed with 45 L of a mixture of concentrated diatoms. The settlement success and the survival of the post-larvae was very poor (less than 1%). Therefore the post-larvae from all three settlement tanks were gathered in one tank in order to lower unnecessary use of resources and workforce. The post-larvae were transferred 10 weeks after the spawning and had a size between 1 and 5 mm at that time (Fig. 14). Each plate was examined and the abalone juveniles were gently scraped off by hand and were brought to stocking densities of 10 individuals per plate. From then on, the post-larvae were fed with 18 L of a mixture of the three species of diatom mentioned above.

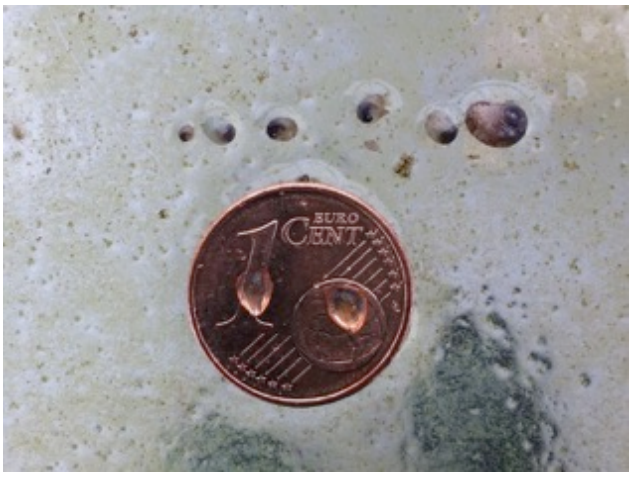

Figure 14. *H. tuberculata coccinea* post-larvae on settlement plate during transfer (Photo: Jesse Theilen 07.06.2017).

3.3 Macroalgae biofilters

3.3.1 Biomass production

During the trial, the biofilters were maintained with variations in water renewal and stocking densities. The data maintained for this section were obtained in a three week section of a trial (24.01.2017 until 14.02.2017). The conditions in this three week period was maintained with

high water exchange and low stocking density of algae (HFLD). Water exchange with 12 renewals of volume per day are resulting in a water flowrate of 20.8 L min^{-1}. The tanks were inoculated with 6.2 kg of *U. rigida* (2.5 g L^{-1}) and 20 kg of *H. cornea* (8 g L^{-1}).

Values for growth and production per day were calculated from the harvested biomass on a weekly base (Tab. 3). The best growth was accomplished in *U. rigida* by Tank 1 during week 2 (9.1 % d^{-1} growth and 75 $gDWm^2$ d^{-1} production) and the worst growth by *H. cornea* during week 3 in Tank 4 (0.2 % d^{-1} growth and 2.3 $gDWm^2$ d^{-1} production) and 2.3 (Tab. 3). In general growth and the associated production was higher in *U. rigida* than in *H. cornea*.

Table 3. Algae biomass production of *U. rigida* (green) and *H. cornea* (red) at HFLD.

		Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6
Week 1	24.01.2017 inoculation (kg)	6.2	6.2	6.2	15	20	20
	31.01.2017 harvest (kg)	10.6	10.3	10,2	16.0	22.3	21.2
	Growth (%d^{-1})	7.7	7.3	7.1	0.9	1.6	0.8
	Production ($gDWm^2$ d^{-1})	60	55.9	54.5	7.8	17.9	9.4
Week 2	31.01.2017 inoculation (kg)	6.2	6.2	6.2	20	20	20
	07.02.2017 harvest (kg)	11.7	11.2	11.3	21.8	22.1	23.3
	Growth (%d^{-1})	9.1	8.4	8.6	1.2	1.4	2.2
	Production ($gDWm^2$ d^{-1})	75	68.2	69.5	14	16.4	25.7
Week 3	07.02.2017 inoculation (kg)	6.2	6.2	6.2	20	20	20
	14.02.2017 harvest (kg)	11.5	10.6	11.5	20.3	21.2	22.2
	Growth (%d^{-1})	8.8	7.7	8.8	0.2	0.8	1.5
	Production ($gDWm^2$ d^{-1})	72.3	60.0	72.3	2.3	9.4	17.1

Total means for each species productivity were calculated for *U. rigida* (growth: 8.2±0.7 %; production: 65.3±7.3 $gDWm^2$ d^{-1}) and *H. cornea* (growth: 1.2±0.6 %; production: 14±6.6 $gDWm^2$ d^{-1}).

3.2.2 Biochemistry analysis of macroalgae

Ash content

Ash, the second biggest fraction has shown a range between 28.5 % and 42.1 %. The ash content was about approximately 10 % higher in *H. cornea* (41.3±0.6) than in *U. rigida* (30.7± 1.6) (Tab. 4).

Table 4. Biochemical analysis of both macroalgae reared in the IMTA system: Ash.

Species	Week	ASH %
U. rigida	1	32
	2	28.5
	3	31.5
H. cornea	1	41.1
	2	42.1
	3	40.8

Protein content

Under the condition of HFLD protein, the third biggest fraction has shown percentages between 14.6 % and 17.3 % (Tab. 5). The average value for *U. rigida* is 15.9±0.8 and for *H. cornea* 15.5±1.3.

Table 5. Biochemical analysis of both macroalgae reared in the IMTA system: Protein.

Species	Week	PROT. %
U. rigida	1	15.9
	2	17
	3	14.9
H. cornea	1	17.3
	2	14.6
	3	14.6

Total lipid content

The lipid fraction displays the smallest fraction with ranges between 2.47 and 3.43 (Tab. 6). The average value for the lipids amounts 3.3±0.1 for *U. rigida* and 2.9±0.4 for *H. cornea*.

Table 6. Biochemical analysis of both macroalgae reared in the IMTA system: Lipids.

Species	Week	LIP %
U. rigida	1	3.3
	2	3.4
	3	3.2
H. cornea	1	3.4
	2	2.5
	3	2.7

Carbohydrates

The biggest fraction displayed by the carbohydrates has shown approximately 10 % higher carbohydrate content in *Ulva* (50±1) than in *Hydropuntia* (40.3±1.5). The values ranges between 38.2 % and 51.1 % (Tab. 7).

Table 7. Biochemical analysis of both macroalgae reared in the IMTA system: Carbohydrates.

Species	Week	CARB %
U. rigida	1	48.7
	2	51.1
	3	50.3
H. cornea	1	38.2
	2	40.8
	3	41.8

4. Discussion

The animals from the different broodstock tanks are of different age and size. The broodstock was fed for conditioning and was not under trial with specific diets and amounts of food. These circumstances prohibit a further interpretation of correlation between the different brood-stock tanks. However, due to the age difference of brood-stock maintained in the facilities certain tanks have shown a better growth than others. Repro 1 for instance showed the best growth performance whereas Repro 17 displayed the worst. Moreover, the feed composition also plays a major role in abalone growth and welfare (Nelson et al. 2002; Viera 2014) as well as the state of development of the specimens. The weight of individuals also vary depending on the state of gonad development and the feed present in the intestine. The gonads of the individuals monitored were under constant development and varied according to the different seasons. Meaning animals were sometimes spawning spontaneously when reaching mature gonad state (GSI 3) and once the animals had released all their gametes they were left with a GSI of 0 again. Even though four tanks had a decline in biomass, the biomass growth of the other tanks has compensated the loss. In terms of brood-stock monitoring and control of juveniles' growth a regular sampling of specimens is required as well as a control of mortality events. Dead animals are always recorded when being sighted. Common causes of death are stress, bacterial or parasite infections or spontaneous spawnings. When spawning, the animals seek for optimal place to release their gametes. Often they end up outside the cages and die because of exhaustion, stress and a lack of hydration. When sighted they were transferred back to a tank by staff members. Due to this animals were sometimes misplaced in different tanks which explains the unexpected increase of animals in Repro 16, Repro 11 and Repro 10.

Studies investigating successful spawning induction methods in abalone are reported in the literature and consist in various options with varying degrees of success. In Moss et al. (1995) the hydrogen peroxide method by Morse et al. (1977) has been applied as an effective method to induce spawning in *Haliotis iris.* However the settlement success was not described in that study. Previous spawning activities in the ECOAQUA-facilities which applied the hydrogen peroxide method have shown also very poor settlement rates (Courtois de Viçose and Viera, oral communication 8.6.2017). In general, such artificial spawning induction causes animals to release not fully developed gametes which has effects on the fertilization rate and also the via-

bility of the offspring what could explain the poor survival observed for *H. tuberculata coccinea*. In general spawning induction events are carried out according to environmental cycles also know to influence natural reproduction cycles such as full or new moon or changes in the ambient water temperature. The ultraviolet spawning method (Kikuchi and Uki, 1974) is a more gentle way to induce the spawning than chemicals and is a methods that has given previous successful results with *H. tuberculata coccinea*.

Once settled the post larvae are then fed different strains of benthic diatoms which nutritional value depends on their physiology (size and shape), digestibility and biochemical composition (Courtois de Viçose et al., 2012c). *Amphora* sp. has high protein and lipid fractions especially during exponential phase (Courtois de Viçose et al., 2012c). *Amphora* sp. also fits in the criteria for post-larvae feed from Hahn (1989), a cell size smaller than 10 µm. *Amphora* sp. has shown superior growth than *N. incerta.* Due to its properties *Amphora* sp. is good feed for abalone post-larvae. Nevertheless due to previous studies it is known that a mixture of more than one algae species enhances growth and health of the post-larvae (Courtois de Viçose, oral communication 06.2017). In order to enhance the post-larvae feeding strategies, once the diatoms begin to attach to the settlement plates the algal growth in the settlement tanks is controlled by using sun covers in order to lower the light incidence and regulate the microalgae growth. The thickness of the algae cue displays an important factor, especially during the first weeks of post-larvae nursery. The light regulation in the post-larval tanks also contribute to shield the photophobic post-larvae from high sun radiation. The two diatoms species have reached the stationary phase after one day of culture. However the data obviously containing errors resulting from bad measuring. The cells numbers are still under high fluctuation after reaching the assumed stationary phase. Anyway, according previous research by Courtois De Viçose (2012c) the stationary phase was reached after day two for both, *Amphora spp.* and *N. incerta.*

In a quest to constantly improve the feed quality provided to abalone, various experiments are undertaken under different production conditions with various type of macroalgae. During one of the experiment running during the traineeship, *Ulva* has shown superior growth than *H. cornea* under similar culture circumstances. Even though the inoculated Ulva biomass was way lower it even maintained more production per day than *H. cornea*. Total *U. rigida'*s production was about 4.5 times higher than *H. cornea*'s production. This shows that the *Ulva* is a more productive species. Moreover, *H. cornea* had to deal with some more procedural issues in the biofilter tanks than the *Ulva* taking into account that *H. cornea* tanks are more prone to be contaminated with other algae species (*Enteromorpha* sp. and *U. rigida*) who grow as epiphytes on the *H. cornea*.

The biochemical composition of the two cultivated macroalgae showed similarities in protein and lipid content. The difference between species in protein content amounts were of 0.5 % and in lipid 0.4 %. In this setup, the biochemical compounds that showed differences are ash and carbohydrate contents. The carbohydrate content in *U. rigida* is 9.7 % higher than in *H. cornea* and the ash content is 10.6 % lower. With a protein content of nearly 16 %, the algae in this setup can be seen as not enriched, since in previous experiments algae with the same

protein content were labeled as not enriched (Viera, 2011). Nutrient enriched macroalgae have been used for previous feeding trials in the facilities. Enriched algae have reached protein contents of 33.76±0.5 % (*U. rigida*) and 29.35±2 % (*H. cornea*) and have also increased lipid amounts (Viera, 2011). The seaweed´s biochemical composition highly depends on the growing conditions as well as seasonal changes (Viera, 2011). In the experiment the algae tanks were also supplied with effluent water from the fish ponds. The much lower protein content results can be linked to a lower ammonium excretion of the captive fish. This can be explained by the fact that during the trial various fish tanks were not stocked and there was a low amount of adult fish kept in the tank because at that time many parts of the facility were occupied with fish larvae production.

5. Conclusion

Modern aquaculture has a huge need for diversification in order to enhance sustainable development. Production of new species, especially those with low environmental impact, has a high importance. Therefore the interest in abalone culture in IMTA is increasing. Abalone production in IMTA still needs to achieve further development. Although great advances have been obtain in the last two decades in abalone production, there is always a need to further research in various directions such as for example, the conditioning, nutrient supply and therefore the algae quality from the biofilters and also quality of larvae and larvae settlement and an optimized alimentation of juvenile post-larvae (diatoms) in order to achieve a more sustainable production. The implementation of IMTA into conventional aquaculture needs to be pursued in the future. Overall, IMTA is regarded as an approach to encourage aquaculture industry to invest into sustainable methods in order to reduce environmental impacts by producing marketable byproducts alongside finfish aquaculture.

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6. Appendix

6.2 Raw data

Table 8. Data from abalone broodstock monitoring. Talla=size; x̄ talla=standard deviation size; peso=weight; x̄ peso= standard deviation weight; sexual maduracion=gonad sexual index; individuos=individuals; biomassa=biomass.

Repro 5 (hembra C)	Octubre 16	Febrero	Marzo	Mayo	Junio	
Talla		68.5	71.1	71.3	72.5	
x̄ talla		7.9	6.7	5.2	5.0	
Peso		52.7	58.7	61.9	62.5	
x̄ peso		15.7	10.6	11.1	10.8	
Sexual maduracion						
x̄ sexual maduracion						
Individuos		5.0	4.0	4.0	4.0	
Biomassa		263.4	234.6	247.4	249.8	
shell growth						30.5
weight gain						18.5
Repro 7 (macho D)	Octubre 16	Febrero	Marzo	Mayo	Junio	
Talla		68.1	68.1	69.0	70.1	
x̄ talla		6.1	5.4	4.2	4.5	
Peso		56.2	59.0	56.0	60.5	
x̄ peso		15.9	16.4	11.2	12.0	
Sexual maduracion			1.8	0.3	1.3	
x̄ sexual maduracion						

Individuos		12.0	12.0	11.0	11.0	
Biomassa		673.9	707.4	616.4	665.5	
shell growth						15.0
weight gain						7.7
Repro 16 (macho S. V.)	Octubre 16	Febrero	Marzo	Mayo	Junio	
Talla		58.8	59.4	61.4	62.2	
$\bar{x}$ talla		3.9	4.3	3.3	3.7	
Peso		33.8	34.2	36.8	35.3	
$\bar{x}$ peso		6.4	6.0	6.3	6.0	
Sexual maduracion		2.3	1.8	1.6	1.1	
$\bar{x}$ sexual maduracion		0.5				
Individuos		44.0	44.0	41.0	43.0	
Biomassa		1487.0	1506.7	1508.1	1518.9	
shell growth						25.6
weight gein						4.5
Repro 11 hembra S. V.	Octubre 16	Febrero	Marzo	Mayo	Junio	
Talla	54.0	58.6	60.8	62.6	63.9	
$\bar{x}$ talla	2.9	4.1	3.8	3.3	2.9	
Peso		34.0	35.0	36.5	40.3	
$\bar{x}$ peso		5.6	5.5	5.1	4.9	
Sexual maduracion		2.5	1.7	1.2	1.4	
$\bar{x}$ sexual maduracion		0.5				
Individuos		30.0	31.0	30.0	30.0	
Biomassa		1020.5	1084.7	1095.6	1207.8	
shell growth						40.5
weigth gain						18.4
Repro 1 (hembra S.V)	Octubre 16	Febrero	Marzo	Mayo	Junio	
Talla	52.3	57.8	59.8	63.2	64.1	
$\bar{x}$ talla	3.2	2.2	3.2	3.1	2.9	
Peso		30.9	35.2	40.8	43.2	
$\bar{x}$ peso		6.0	4.8	5.5	5.4	
Sexual maduracion		2.0	1.4	1.5	1.4	
$\bar{x}$ sexual maduracion		0.4				
Individuos		18.0	18.0	17.0	16.0	
Biomassa		555.7	633.9	693.2	690.7	
shell growth						48.5
weight gain						39.8
Repro 13 (hembra E)	Octubre 16	Febrero	Marzo	Mayo	Junio	
Talla	52.8	59.8	61.7	63.1	64.4	
$\bar{x}$ talla	2.0	2.6	2.4	2.5	2.3	
Peso		35.3	36.9	37.1	40.4	
$\bar{x}$ peso		4.9	5.4	5.0	6.7	
Sexual maduracion		2.3	1.8	1.2	1.1	
$\bar{x}$ sexual maduracion		0.4				
Individuos	30.0	18.0	18.0	18.0	18.0	
Biomassa		634.6	664.4	667.6	727.1	

shell growth						35.2
weight gain						14.6
Repro 14 (hembra D)	Octubre 16	Febrero	Marzo	Mayo	Junio	
Talla	71.5	62.3	67.0	70.3	76.0	
$\bar{x}$ talla	4.5	3.5	5.3	3.9	0.0	
Peso		51.7	55.1	54.6	76.8	
$\bar{x}$ peso		8.8	11.1	12.3	0.0	
Sexual maduracion		1.7	1.3	54.6	1.0	
$\bar{x}$ sexual maduracion		0.2				
Individuos		3.0	3.0	3.0	1.0	
Biomassa		155.1	165.2	163.8	76.8	
shell growth						104.3
weight gain						48.5
Repro 12 (macho E)	Octubre 16	Febrero	Marzo	Mayo	Junio	
Talla	53.8	61.5	62.5	63.2	66.6	
$\bar{x}$ talla	1.8	1.9	1.5	1.7	1.5	
Peso		34.1	34.5	37.6	44.6	
$\bar{x}$ peso		3.5	3.6	2.7	3.1	
Sexual maduracion		1.3	1.1	1.2	1.4	
$\bar{x}$ sexual maduracion		0.6				
Individuos	30.0	24.0	23.0	21.0	19.0	
Biomassa		819.0	793.7	789.7	847.2	
shell growth						39.0
weight gain						30.7
Repro 8 (macho A+.91+ hembra A44)	Octubre 16	Febrero	Marzo	Mayo	Junio	
Talla	71.6	75.6	75.6	75.8	77.4	
$\bar{x}$ talla	5.8	6.8	6.0	5.3	5.8	
Peso		71.6	77.0	73.1	69.4	
$\bar{x}$ peso		22.3	19.7	18.3	27.8	
Sexual maduracion		1.5	1.3	0.6	0.8	
$\bar{x}$ sexual maduracion		0.8				
Individuos		12.0	11.0	11.0	10.0	
Biomassa		859.6	847.1	804.2	694.3	
shell growth						13.9
weight gain						-3.1
Repro 17 (macho C,B2)	Octubre 16	Febrero	Marzo	Mayo	Junio	
Talla	66.6	67.6	67.5	67.3	68.7	
$\bar{x}$ talla	4.7	4.8	5.0	4.8	5.1	
Peso		49.9	51.0	50.7	50.8	
$\bar{x}$ peso		11.5	11.6	11.4	11.3	
Sexual maduracion		1.5	1.1	0.7	0.7	
$\bar{x}$ sexual maduracion		0.7				
Individuos		28.0	28.0	28.0	28.0	
Biomassa		1396.3	1427.5	1418.6	1421.6	
shell growth						8.5

weight gain						1.8
Repro 3 (macho S.V)	Octubre 16	Febrero	Marzo	Mayo	Junio	
Talla	52.5	61.1	62.4	63.3	65.3	
$\bar{x}$ talla	2.9	3.6	3.6	3.9	65.3	
Peso		38.0	40.6	39.0	45.8	
$\bar{x}$ peso		5.3	5.9	6.1	45.8	
Sexual maduracion		2.3	1.8	1.5	1.9	
$\bar{x}$ sexual maduracion		0.3				
Individuos		23.0	23.0	23.0	22.0	
Biomassa		875.0	933.7	897.5	1006.6	
shell growth						31.8
weight gain						20.3
Repro 2 (hembra S. V.)	Octubre 16	Febrero	Marzo	Mayo	Junio	
Talla	51.2	58.4	59.8	60.5	62.4	
$\bar{x}$ talla	2.2	2.7	3.0	2.5	3.0	
Peso		32.1	33.7	32.8	37.7	
$\bar{x}$ peso		5.6	5.7	4.9	6.0	
Sexual maduracion		1.8	1.4	1.0	1.4	
$\bar{x}$ sexual maduracion		0.4				
Individuos		30.0	30.0	27.0	27.0	
Biomassa		962.7	1012.4	886.8	1019.1	
shell growth						30.9
weight gain						17.6
Repro 10 (macho S. V.)	Octubre 16	Febrero	Marzo	Mayo	Junio	
Talla		60.5	61.1	62.4	65.6	
$\bar{x}$ talla		3.1	3.5	4.1	5.2	
Peso		38.3	34.0	37.3	44.5	
$\bar{x}$ peso		6.4	6.3	7.0	6.7	
Sexual maduracion		2.4	1.2	1.3	4.2	
$\bar{x}$ sexual maduracion		0.5				
Individuos		27.0	23.0	24.0	23.0	
Biomassa		1033.8	782.7	894.0	1024.0	
shell growth						38.5
weight gain						16.3
Repro 15 (hembra S.V)	Octubre 16	Febrero	Marzo	Mayo	Junio	
Talla		63.8	65.6	67.5	69.2	
$\bar{x}$ talla		3.4	3.7	4.3	4.4	
Peso		42.0	43.9	47.3	49.3	
$\bar{x}$ peso		7.7	8.2	10.6	10.3	
Sexual maduracion		2.0	1.6	1.4	1.2	
$\bar{x}$ sexual maduracion		0.5				
Individuos		23.0	23.0	21.0	20.0	
Biomassa		966.5	1009.7	994.1	986.7	
shell growth						41.7
weight gain						17.4
Repro 18 (hembra S. V.)	Octubre 16	Febrero	Marzo	Mayo	Junio	

Talla	53.8	61.7	63.4	64.4	65.7	
$\bar{x}$ talla	7.4	1.9	2.2	2.2	2.6	
Peso		39.3	40.3	42.5	45.4	
$\bar{x}$ peso		4.2	4.7	5.0	5.8	
Sexual maduracion		2.0	1.6	1.3	1.4	
$\bar{x}$ sexual maduracion		0.4				
Individuos	26.0	24.0	24.0	24.0	22.0	
Biomassa		942.9	967.7	1020.1	997.9	
shell growth						30.2
weight gain						15.5

Table 9. Monitoring data from 30 tagged animals. Females (Tarima 1)

February	size	weight	June	size	weight
E104	58	27.7	E104	63.5	35.7
E109	59	30.2	E109	62.5	34.9
E140	51	18.7	E140	54	21
E108	48	16.5	E108	51.5	20.6
E192	49.5	17.8	E192	52.5	29.4
E167	48	18.3	E16	59	35.2
E142	53	25.5	E142	59.5	30.3
E37	46.5	15.3	E37	48	15.3
E17	50	25.6	E17	53.5	25.2
E128	47	19.1	E128	53	21.2
E181	55	28.7	E181	61.5	32.4
E63	54	27.8	E63	56.5	32.4
E62	53	27.6	E62	56	31.3
E114	54.5	25.6	E114	58.5	28.9
E6	54	29.1	E6	58	26
E93	52	22.8	E93	57.5	27.5
E128	47	19.1	E128	51.5	19.5
E71	52	24	E71	57.5	28.4
E18	48	19	E18	50.5	20.9
E117	53	21.8	E117	57	22.4
E120	52.5	20.7	E120	57.5	26.6
E179	49.5	19.6	E179	45	34
E76	45	25.1	E76	61	31
E137	48.5	17.8	E137	54	21.9
E65	52.5	24.6	E65	56	25.3
E14	45.5	27	E14	58.5	33.7
E58	56	27.4	E58	62	35.5
E176	48.5	21.3	E176	53.5	23.6
E92	58.5	28.5	E92	62	31.5
E157	48	16.4	E157	53.5	23.9
E86	52	22.3	E86	54	27.6
Average	51.25806	22.93226		56.08	27.52

Standard deviations	3.72626	4.358632			4.32	5.43

Table 10. Monitoring data from 30 tagged animals. Males (Cestas verdes)

February				June		
E35	62	38.3		E35	68.5	45.7
E70	54.5	22.9		E70	62.5	29.7
E38	58.5	32.7		E38	64	40.3
E15	52	21.7		E15	55	25.9
E1	55	27.8		E1	60.5	36.1
E34	54.5	24.9		E34	59	29.3
E60	56.5	28.7		E60	61	33.3
E65	54	29.4		E65	59.5	35.4
E68	60	30.8		E68	66	38.7
E95	65	43.2		E95	70	53.9
E94	59	35.7		E94	65	44.4
E25	55.5	32.4		E25	60.5	39.7
E45	62	42.2		E45	57.5	29.4
E84 red	55	29.7		E84	60.5	34.2
E40	56	29.1		E40	61.5	34.4
E12	58.5	30.3		E12	61.5	39.9
E26	53.5	20.5		E26	56.5	23.1
E69	52	23.8		E69	57	29.3
E47	58	30.9		E47	60	33.1
E37	55.5	32		E37	61	36.7
E59	59	31.5		E59	62.5	39.7
E63	61	39		E63	68	47.1
E20	58.5	32.8		E20	63	38.5
E83	56	27.6		E83	60.5	31.1
E82	50	17.5		E82	55	20.6
E32	54	25.4		E32	60	32.6
E42	59	34.1		E42	64	43.8
E27	58.5	31.4		E27	62.5	37.1
E64	61	36.4		E64	64.5	41.9
E72	58	30.5		E72	63.5	36.9
Average	57.07	30.44			61.68	36.06
	3.34	5.93			3.61	7.11

Table 11. Cells numbers ascertained by Neubauer method of the diatom species *Amphora spp.* and *Navicula incerta*

Day	*Amphora spp.* [$1*10^6$ cells]	*Navicula incerta* [$1*10^6$ cells]
0	0.1	0.1
1	2.3	1.25
2	3.38	1.76
3	5	1.24
4	1.93	1.69
5	2.15	1.45
6	4.03	1.9
7	5.4	1.1