

# Relationships between growth, physiological and behavioural traits of the European abalone Haliotis tuberculata in the perspective of a selective breeding plan implementation

Sébastien Lachambre

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AGROCAMPUS OUEST CFR Rennes 65 rue de Saint Brieuc 35000 Rennes FRANCE HALIOTIS Kerazan 29880 Plouguerneau

Mémoire de Fin d'Etudes

Stage financé par la région Bretagne

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Année universitaire : **2013.2014** Spécialité : Halieutique Spécialisation ou option : AQUA

Relationships between growth, physiological and behavioural traits of the European abalone *Haliotis tuberculata* in the perspective of a selective breeding plan implementation.

## Sébastien Lachambre



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# Résumé

Après dix ans de production d'ormeaux en mer, France Haliotis a le projet de mettre en place un plan de sélection pour diminuer ses coûts de production et maximiser l'utilisation de la diversité génétique de l'ormeau européen Haliotis tuberculata. Un plan de sélection massale semble être adapté à la taille de l'entreprise. Dans les différents pays producteurs, la faible croissance et la mortalité sur des stades de productions avancées sont identifiées comme les deux plus importantes limites aux profits des fermes halioticoles. La croissance est cependant le principal trait amélioré et étudié dans les différents plans de sélection génétique sur l'ormeau. En effet la mortalité liée à la sensibilité de l'ormeau aux agents stressants et aux agents pathogènes (du type Vibrio) ne peut être améliorée directement en sélection massale à faible coût car un challenge bactérien entraine un très fort taux de mortalité. Si les premiers cycles de sélection montrent des résultats encourageants, l'investissement en génétique doit être rentable à moyen et long terme pour l'entreprise. Pour rendre durables les profits attendus, il semble important de pouvoir contrôler la perte de diversité génétique et le risque d'apparition de trade off. Avec un animal à cycle long, une stratégie simple pour répondre à ces objectifs en sélection massale est de sélectionner à l'aide de plusieurs critères de manière simultanée. Les coûts importants de phénotypages et de génotypages sur chaque critère de sélection constituent une limite de cette stratégie pour une entreprise comme France Haliotis. L'objectif de l'étude était donc d'étudier les relations existantes entre des traits de croissance, de physiologie et de comportement, afin d'en sélectionner un nombre restreint parmi les plus intéressants pour la sélection.

Dix-sept traits phénotypiques ont été mesurés sur 120 ormeaux (3 répétitions, 40 ormeaux par répétition) dont la moitié était issue de l'élevage et l'autre moitié était sauvage. Afin de simuler les conditions rencontrées en élevage, des agents stressants rencontrés en élevage (sur(densité et manipulation) ont été appliqués pendant 3 semaines en laboratoire. Puis les ormeaux ont été remis en mer et la croissance a été mesurée après deux mois. Le rythme d'activité des ormeaux a été suivi pendant 48h après une habituation de 5 jours. Des épreuves de réactivité comportementale (épreuve du toucher et du retournement) ont été réalisées au cours des différentes phases d'application des agents stressants. Le nombre total de cellules et l'efficacité phagocytaire ont été mesurés à l'aide d'un prélèvement d'hémolymphe réalisé à la fin de la période de stress. L'analyse des relations existantes entre ces différentes variables a été faite pour les variables qualitatives à l'aide d'une analyse en composante multiple. L'effet de certains seuils pour des traits physiologiques et comportementaux sur la croissance a pu être testé par des tests d'analyse de variance non paramétrique.

L'analyse a montré que la croissance était liée positivement au développement gonadique et à la durée moyenne de mouvement par jour. En revanche, elle était liée négativement à l'efficacité phagocytaire et à la latence de retournement lorsque l'ormeau est placé sur le dos pour l'épreuve de retournement. Une relation semble exister entre un échec à l'épreuve de retournement à la suite des trois semaines d'application de l'agent stressant et la mortalité lors de la période d'élevage. Les ormeaux dont le pied n'était pas rétracté sous la coquille et qui ont bougé lors de l'épreuve du toucher ont eu une meilleure croissance. Ces résultats montrent que des traits comportementaux peuvent être reliés à la croissance et à la mortalité. Les variables quantitatives de l'analyse n'ont pas permis de séparer des profils particuliers d'ormeaux, ni de discriminer de manière claire les animaux morts des survivants, ou les animaux sauvages des animaux élevages. A l'inverse, les variables qualitatives permettent une meilleure discrimination.

Cette expérimentation nous a permis de montrer que les animaux bougeant le plus étaient les animaux ayant une croissance plus importante, probablement lié au fait que ces ormeaux accèdent le plus à la nourriture en cage. A contrario, les ormeaux ayant un comportement de fixation sur leur support semblent être les ormeaux ayant une gestion du stress plus mauvaise, avec une moins bonne croissance et plus de mortalité. Le test de retournement pourrait être un bon indicateur de l'état physiologique de l'animal. Un ormeau se retournant vite disposerait de plus d'énergie, lui permettant de réaliser une croissance plus importante et ainsi mieux résister aux conditions d'élevage en mer.

Il serait intéressant d'étudier l'héritabilité de ces caractères et de voir si les corrélations phénotypiques observées dans cette étude sont liées à des corrélations génétiques. Cette étude montre également qu'une sélection sur la croissance pourrait entraîner une augmentation du développement gonadique et une diminution de l'efficacité phagocytaire, phénomènes tous deux associés à des taux de mortalités plus importants. Il serait intéressant de définir des valeurs moyennes de référence pour les populations d'élevage et sauvage non sélectionnées et de suivre son évolution aux cours des premiers cycles dans le but d'anticiper de tels trade off.

En ce qui concerne les traits comportementaux, des héritabilités faibles sont attendues. Il serait tout de même intéressant d'acquérir des estimations de paramètres génétiques sur ces traits en cas d'héritabilité non nulle. Ces traits pourraient être utilisés pour assister la sélection sur la croissance. Il serait intéressant de voir si les tests comportementaux pourraient être capables d'évaluer les potentiels de croissance des animaux précocement, afin de pouvoir sélectionner des mâles de deux ans et de les croiser avec des femelles de 3 ans pour gagner 30% de vitesse de croissance.

La population d'ormeaux sauvages et la population d'ormeaux d'élevage ont pu être discriminées grâce aux variables qualitatives comportementales et aux traits de croissance. Les ormeaux d'élevage ont eu une plus forte croissance, moins de mortalité, plus de succès au test de retournement et ont bougé plus longtemps par jour en moyenne. Il semblerait que les conditions environnementales présentes lors des premiers stades de développement de l'ormeau ont un impact sur son comportement. Il serait intéressant d'étudier les impacts de ces changements sur la survie des ormeaux juvéniles qui seraient utilisés pour le repeuplement afin d'adapter les pratiques d'élevage ou de sélectionner des animaux ayant un comportement plus adéquat plus efficace pour le repeuplement.

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# Liste des abréviations

F.H : France Haliotis
VGI : Visual Gonad Index
IFREMER : Institut Français de la recherche pour l'exploitation de la mer
SMEL: Syndicat mixte pour l'équipement du littoral
FAO : Food and agriculture organization
GMA: Galician Marine Aquaculture

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# **Avant Propos**

Mon stage s'est déroulé du 10 février au 15 août. Il a été réalisé en 3 étapes :

- la collecte de données dans l'exploitation de France Haliotis à Plouguerneau (du mi-février à mi-mars)
- l'analyse des vidéos et l'analyse statistique dans le département de zoologie sous la responsabilité du chercheur Rob Day, spécialisé en écologie de l'ormeau (Université de Melbourne, Australie) (de mi-mars à mi-juillet)
- la rédaction du rapport et la préparation de l'oral à Plouguerneau (mijuillet à mi-août)

Même si la réflexion produite lors de ce stage a été conduite dans la perspective de l'établissement d'un programme de sélection à France Haliotis, cette expérience ne représente qu'une étape préliminaire. L'acquisition de connaissances fondamentales en biologie sur l'ormeau est nécessaire pour la réalisation ultérieure d'un programme de sélection durable. L'objectif de ce rapport est donc de présenter les relations phénotypiques existantes entre différents traits mesurés sur 120 ormeaux bretons *Haliotis tuberculata*.

Le travail a été commandité et financé par Sylvain Huchette, directeur de France Haliotis. L'encadrement scientifique a été réalisé par Sabine Roussel, enseignante-chercheur au LEMAR. Un étudiant de M1, Antoine Rio-Cabello, a également participé à la collecte des données.

Le mot « abalone » est invariable et ne prend pas de « s » au pluriel, sauf lorsqu'il s'agit de plusieurs espèces d'ormeaux.

# Introduction

## A. Abalone production

#### A.1. Worldwide abalone production

Abalone (Haliotis sp.) have been consumed in Asia for centuries as a part of traditional food preparation and ceremony (Francis et al. 2008). They are also consumed in Europe and currently represent high market values (between 30 to 100 euros/Kg in France). Worldwide demand exceeding its supply, it resulted in an excessive pressure on the stocks in many fisheries (Gordon & Cook 2004). Consequently abalone wild stocks have continued to decrease all over the world (Huchette & Clavier, 2004; Rhode et al 2012). Fishing production is now strongly regulated and limited by licenses in many fisheries and seems to be stable at around 12000 MT (FAO 2012, Figure 1).

At the same time, an increase of aquaculture's production was observed. Considering the high incertitude regarding Chinese production, it's difficult to estimate a worldwide aquaculture production. Nevertheless, production doubled between 1990 and 2000 to raise 3500 MT (Elliott et al. 2000). This increase continued between 2000 and 2014 and aquaculture supply is now much higher than fisheries production (FAO 2012).

Current aquaculture production is estimated at 100000 MT and is supported at 97% by Asia (China, Japan, Hong Kong). The other producer countries are South Africa (1000MT), Australia and New Zealand (800MT), United States and Chile (1000MT) (FAO 2012).



**Evolution of Abalone productions** 

#### A.2. French abalone production

France is a small producer country with around 20MT produced by fishing (Basuyaux et al. 2001) and 8 MT produced by three aquaculture farms (Huchette, pers com). Only one

Figure 1: Evolution of worldwide abalone production (Source : FAO 2012)

*Haliotis* species *Haliotis tuberculata* is naturally present in Europe. The first experimental production and technical studies were led by IFREMER in the late 1970s and early 1980s (Flassch et al. 1984). They developed techniques for rearing, conditioning, and spawning. Later on, these techniques were perfected by the SMEL (Nicolas *et al.* 2002). Their recent work also included preparation of broodstock for reproduction (inversion of photoperiod cycle for spawning during daytime and reduction of the conditioning cycle to 1 - 2 months) (Basuyaux *et al.* 1999). Despite a long production cycle between 3.5 and 4 years, 3 land-based abalone breeding companies were created in Normandy and Vendee in 2001 producing small cocktail-size abalone to supply gastronomy restaurants.

France Haliotis activity began in 2004 on the North West Brittany coast using a sea growing production system with nursery practices developed in Australia. Sea production opportunities, large nurseries capacity and important applied research studies quickly led France Haliotis to be the biggest farm in France with 7 metric tons of annual production. Today 2 others growing farms of *Haliotis tuberculata* are installed in France making half-growing and buying juveniles to France Haliotis. In Europe, an Irish farm Tower Aqua and Spanish farm GMA financed by a European project were created. Large recirculation systems were built for industrial production. Despite extensive knowledge of the issues associated with the movement of foreign species, these farms were allowed to produce *Haliotis discus hanaii*, a Japanese abalone. After buying many juveniles to France Haliotis company, the Spanish farm never succeeded raising *Haliotis tuberculata* and is actually focusing its activity in *Haliotis discus hanaii*.

#### **B.** Haliotis tuberculata, a european gastropod with slow growth

*H. tuberculata* is a marine gastropod which lives in the low intertidal and subtidal zone in rocky areas. Younger animals seem to prefer shallow areas (Clavier and Richard 1985). It's exclusively present in Europe between North Africa and the south of the United Kingdom. Two sub classes were described: *H. tuberculata tuberculata*, and *H. tuberculata coccinea* (Figure 2). Modern classification of common animal taxa based on molecular and morphological evidence shows that *Haliotis sp.* are closer to cephalopod than bivalves (Figure 3).



Figure 2 : Distribution of *Haliotis tuberculata: H. tuberculata tuberculata* is represented in red, and *Haliotis tuberculata coccinea* in blue (Source : Huchette et Clavier 2004)



Figure 3: Simplified phylogenetic tree of Protostomes (Source: Hooper et al. 2007)

*Haliotis tuberculata* is a gastropod with one shell which covers him completely (Figure 4). Like many gastropods, it perceives its environment through cephalic eye and foot tentacles. The foot allows abalone to crawl and stick strongly to a solid substrate and represents around 34% of the total weigh mass for 2.5 years old animals (personal data). The shell is pearly and has a potential market value (Figure 4).



Figure 4 : A) Ventral view B) Facial view C) Dorsal view and D) shell (Source : Travers et al. 2008)

Abalone are nocturnal opportunistic feeders and can accept a large diversity of diets in the wild (Troell *et al.* 2006) but some studies have shown that there are able to choose their

diets (Flemming *et al.* 1995). The two most significant criteria of choice seem to be the thickness of the algae and his nutritive value (Roussel, pers. comm.). Gastropods are known to have olfactory and gustatory receptors and are considered to find their foods by chemoreception (Croll, 1983). The presence of small algae particles in tanks raised the consummation rate on artificial pellets (Allen 2006). Its radula (Figure 4A) allows it to graze algae.

In Brittany, wild reproduction occurs in August when water temperatures reach 16°C. The abalone is gonochoric: females are recognized with a dark color gonad while the males have a clear one (Figure 4 B). In the wild, a sex ratio close to 1:1 is observed (Cochard and Flassh 1981). Abalone has a R reproductive strategy (production of a large amount of zygotes with high mortalities): a single female can produce over 2 millions viable gametes (personal data).

Abalone are very sensitive to any change of their environment and to stressors. Pathogens have induced high episodic mortalities in the wild (Travers *et al.* 2008). On farms, a simple manipulation can also enhance mortalities up to 40% (Huchette, pers. comm.; Malham *et al.* 2003; Cheng *et al.* 2004; Hooper *et al.* 2011). Despite its sensitivity and slow growth (4 years to reach adult size), the first steps of domestication of *Haliotis sp.* have occurred in different part of the world.

#### C. A species at the beginning of the domestication process

Domestication is a "long process by which a population of animals becomes adapted and dependent to humans and to a captive environment by some combination of genetic changes occurring over generations and environmentally induced by events reoccurring in each generation" (Price, 1984). It can also be defined by the rearing of a small population with the loose of panmixia (Random mating with the entire wild population) for which the natural selection pressure is suppressed (Helmer 1992). The first species domesticated is the wolf with a domestication process taking place 15 000 years ago (Vigne 2011; Mazoyer and Roudard, 2010).

The early domestication process induced several changes in the domesticated population such as a lower reaction to density stressors (Kunzl and Sachser, 1999) and increased feeding behaviour (Gustafsson *et al.* 1999; Andersson *et al.* 2001) in domesticated pigs, a reduced fear responses in presence of predators of pigs (Yamamoto and Reinhardt, 2003). These behavioural changes are associated with physiological changes such a decrease of cortisol and catecholamine hormones after the stress application in guinea pigs (Kunzl and Sachser, 1999) Domestication generally induced the reduction of the size and a diminution of aggressiveness in large species but an increase of size in small species (Vigne 2012). Even if genetic had a key role in these change, they can also be linked with the fact that farm population grew up in a really different environment during ontogeny compared to wild population.

It's also well documented that massal selection speeds the rate of domestication which can increase indirectly the welfare of animals. It was already described for the fish (Vandeputte and Prunet 2002). This fact can be explained by the higher nutrient availability and the protection against predators that supply the farm environment. Nevertheless in the last 50 years, the selection is getting faster with a success of breeding plan explained by more accurate estimations of breeding value and moderate to high heritability for production traits selected (Rauw *et al.* 1998). However, selection of animals for high production efficiency based most of the time on one or few traits seems to submit animals to behavioural, physiological and immunological higher disorder risks (Rauw *et al.* 1998).

In France Haliotis, the total abalone rearing cycle is controlled (Figure 5). In contrast to wild abalone, farm population grow up without natural selection pressure, with no or few predators, an high food availability, in an artificial cage rearing environment with high density (40 abalone / m2). Consequently, some phenotypic differences in juvenile stage can already be observed such as the shell color due to *ulvela* feeding in juvenile stage and foot more developed in contrast to wild abalone (Huchette, pers. Comm.). France Haliotis company started 10 years ago, which correspond to the realization 3 production cycles. However, abalone reared on farm are submitted to specific stressors related to husbandry practices (Figure 5). High density, manipulation such as shaking and detachment, pathogen development, low water quality are some of the stress experienced regularly by farmed abalone (Malham *et al.* 2008, Cheng *et al.* 2005).



#### Production cycle of Fance Haliotis, stress and selection induced

Figure 5: Production cycle of *Haliotis tuberculata* in France Haliotis with different stressors (Red), target (Green) and untargeted selection (Blue) linked with farm practices

Target and untarget selection can happen during the production cycle: for example untargeted selection to husbandry practices happens at the settlement period or after shaking and target selection is observed when abalone are calibrated at juvenile stage and at the end of the production cycle. However it can be hypothesized that no lost of diversity had occurred in France Haliotis due to reproduction strategy consisting in mating each farm abalone selected for reproduction with a wild abalone. In addition, the contribution of wild genitors is probably higher than 75 % due to higher gamete production of wild broodstock (between 1 to 3 million gametes) compared to farm broodstock selected from the larger individuals in cages (between 500 000 to 1 million gametes). This situation allows us to think that France Haliotis is only at the very beginning of the domestication process.

#### D. Genetic selection, the next step to improve abalone farming

#### **D.1. Selective breeding plan**

*Haliotis tuberculata* growth is slow (3.5 to 4 years to reach the commercial size), inducing a high production cost and increasing the risk of accident during the production cycle. Food distribution in France Haliotis is based on fresh algae collected from the wild. No storage of this resource is possible. The nutrition is totally dependent of wild algal production and quality, collected on area certified for organic farming, and submitted to variable meteorological conditions. Another difficulty is the important mortalities in the summer at final production stage which seems to be linked to a combination of pathogen activity in warm temperature (*Vibrio harveiy* 17°C), animal mature reproduction status, and stress caused by feeding or manipulation (Travers *et al.* 2007). In this global context, it's difficult for the company to create sustainable strong production profit.

In order to increase its benefits, a manager of an aquaculture farm can usually work with 4 solutions: the production size, the management and zootechnical process, the nutrition and the genetic (Elliott 2000). After some zootechnical and nutrition applied research program (SUDEVAB, IDEALG), France Haliotis wants now to lead a selective breeding plan to go further in the domestication process and increase his profit.

Two questions need to be answered before the implementation of a selective breeding plan:

- 1) In what population will we work?
- 2) What traits do we want to improve?

To answer to the first question, France Haliotis has two choices: use the current farm population and benefit from the early domestication process which was realized over the past 10 years, or starting with a new large and diverse wild population to maximize the sustainability and the potential of the selective breeding plan. This question will not be addressed in this study but the choice strongly depends on the effective population size and on the level of homozygosis already present in the farm population. These two parameters will need to be measured prior to the realization of a selective breeding plan.

To answer to the second question, two issues need to be addressed: minimize the loose of genetic diversity and increase production gain. Desirable traits need to be targeted according to the economic gain and manager France Haliotis farm objectives.

France Haliotis has developed two market products: live abalone sent to gourmet restaurants, and frozen eviscerated abalone sold to a wholesaler specialized in frozen food. The summer mortality in final stage of production and the low growth are identified to be the bigger break to make profits. In this context, four traits would need to be improved in order increase the farm economic efficiency (Table 1): growth rate, meat yield at commercial size, resistance to pathogens and reduced responses to farm stressors.

Another market in development for France Haliotis is the sea-ranching in association with fisherman. The worldwide low surviving rate of ranching program may be partly explained by changes in the fitness of farm populations reared in nursery facilities during ontogeny as well as the choice of the ranching site (Roodt-Wilding *et al.* 2007). In addition, juveniles may present non-adapted behavioural responses to predator due to the lack of exposition during ontogeny. In the wild, juveniles with nonadapted behavioural responses to predator will probably be eaten and will not reach reproduction stage, or only if their environment is predator-free. The changes of behaviour between wild and farmed abalone due to domestication process need to be studied further with regards to sea ranching activities.

r		
	Trait to improve	Observation of the trait
1	Growth rate from juvenile stage to	Specific growth rate of abalone individually
	commercial size with algae diet in sea-cage	tagged
	rearing	
2	Meat yield weight at commercial size	Possibility 1 : Condition index (CI)
		calculated to measure the ecophysiological
		state of abalones as: CI=W/L^3 with the
		length (L) in cm and the weight (W) in g
		(non invasive measure)
		Possibility 2 : muscle on total weight ratio
		(invasive measure)
3	Multi bacterial resistance during summer	Possibility 1: non-invasive proxy (need to be
	(Mostly Vibrio families)	found)
		Possibility 2: bacterial challenges applied to
		the broodstock and selection of the resistant
		broodstock (invasive)
4	Reduced responses to farm stressors	Need to find non-invasive proxy
	(Shaking, high density, take-off support	(physiological or behavioural tests)
	structures, movement, immersion, light	
	stress)	
	,	

#### Table 1: List of the main economic traits in order of importance

#### **D2.** Choice of criteria for selection

A trait which can be improved in a massal selective breeding plan is a phenotypic trait easy and cheap to measure, repeatable, non-invasive, and heritable (Elliott *et al.* 2000). It should allow production gain or lower production costs and give more stability to the production (Table 1).

Length and weight growth rates are easy to measure and moderately heritable. They are most of the time the first target of a selective breeding plan (Gerber et al. 2013; Lucas et al. 2006). We assume that the growth rate from larvae settlement to commercial size is an integrative measure of disease resistance and stress resistance to husbandry practices.

Several physiological traits would be interesting to follow. However, most of them are time-consuming and costly to measure. Total haemocyte count and phagocytosis efficiency were measured during this experiment. There are good indicators of immunologic efficiency, especially phagocytosis (Hooper *et al.* 2007; Travers *et al.* 2008) and are affected by many farm stressors (Cheng *et al.* 2004; Malham *et al.* 2003; Hooper *et al.* 2011; Hooper *et al.* 2014). However, the main disadvantages are there invasiveness, leading to an increase of mortality due to handling and haemolymph sampling.

Behaviour can be good complementary tools to follow changes in the homeostatic balance of animals and provide direction of domestication process. The first steps of the domestication process for many species were clearly more related to behavioural traits than to production traits (Vigne 2011; Mazoyer and Roudard 2010). *Haliotis tuberculata* is characterized by two patterns of personality with "wanderers" abalone moving, occupying hide and showing a more pronounced homing behaviour than "sedentary" abalone (Cenni *et al.* 2010). Abalone selected for a better growth realized more mouvmentthan others (Werner *et al.* 1995; Robinson *et al.* 2010).

Relationships between behavioural reactivity of the abalone, 24-h activity, feeding behaviour and growth performance are still unknown. In addition, these relationships may differ according to the rearing environment such as high density, repeated handling, etc... Strong individual variability is observed which lead to assume good capacities of improvement if a selection were realized in these traits. Behavioural traits can be heritable as shown for cephalopods, a mollusk group close to the abalone (Sinn *et al.* 2005; Sinn *et al.* 2006; Hooper *et al.* 2007). However, heritability of behaviour is still unknown in abalone. Behavioral trait might be non-invasive integrative proxy of a good efficiency in farm. However, these variables can be difficult to integrate in selection program due to time necessary to analyze them and difficulties for standardization. For mammal productions they are now included in breeding selection program for example, with sow improved maternal behaviour or decreased response to stress.

## **E.** Implement a selective breeding plan in order to increase production gain

A selective breeding plan of animals with long rearing cycle is an investment on medium term which needs to be planed and related with precise traceability. This investment can be secured by the expected productivity gain. This productivity gain can be estimated by genetic knowledge like heritability or genetic correlation. The heritability of a trait  $(h^2)$  is the proportion of phenotypic variation in the population that originates from genetic variability (Ernande *et al.* 2003). When an heritability is higher than 0.2, it can consider that the traits are worthwhile for a selective breeding plan (Haffrey pers. comm.). Traits such the growth or the meat yield have usually a higher heritability than traits strongly impacted by the environment such as reproductive performance.

As growth is identified to be the most important criteria in making a profit in the abalone industry (Elliott 2010), many studies have been conducted on shell length heritability. Growth length heritability was estimated at 0.36 for *H. asina* (Lucas 2006), 0.3 for *H rufescens*, 0.26 for *H.diversicolor* (You *et al.* 2010), 0.23 *H.discus hannai* (Deng *et al.* 2007). A preliminary experiment on *H. tuberculata* shell length showed an heritability of 0.37 (Roussel *et al.* 2010). The improvement of growth performance can be substantial with an increase of length growth rate of 50% and 65% respectively for *H. rufescens* and *H discus hannai* at the third generation (Elliott 2000).

Three different types of selective breeding program are usually applied in farmed animals, each of them with advantages and inconveniences (Table 2).

Selection type	Principles	Advantage for F.H	Disadvantage for F.H
Massal selection	Selection based on individual performance	Simple, low cost	Risks of trade off and loss of diversity
Familial selection	Selection based on family performance	Precise and works even if h <sup>2</sup> <0.3. Possibility to control inbreeding	High cost to maintain families Confusion effect between family and tank
Genealogic selection	Selection based on synthesis of generation performance	Direct access to a precise genetic value of an animal	Needs a genealogical performance history from several production cycles

Table 2: Main selective breeding approaches (F.H=Fr	ance Haliotis)
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These strategies have already been implemented by different aquaculture productions and abalone farms. As the domestication of abalone is recent, halioticulture has great potential to design more efficient and sustainable selective breeding plans similar to those developed by other agronomic industries (Rhode *et al.* 2012). In particular French production can already learn of the issues experienced in other abalone producing countries (Rhode *et al.* 2014).

One of the first risk, especially when selection is based on one production trait, is the risk of trade-off. Trade-off can be defined as deleterious effect of the selection in untargeted trait which will affected the performance on the selected trait (Rauw *et al.* 1998). These trade-off are extensively described in mammal production such as the reduction of fertility in dairy cattle selected for high milk production (Hoekstra *et al.* 1994). In aquaculture, some trade-off are also observed such as heart failure of salmon selected only on better growth (Torude *et al.* 2004). An explanation of these trade-off are the loose of homeostatic balance in the animal biology due to too fast evolution of one trait or genetic correlations between trait improved and other deleterious traits. The loose of balance is also most of the time associated with high nutrition intake, high density, increasing pathologic pressure and trade-off associated with some selected trait (Rauw *et al.* 1998). The acquisition of extensive data on the biology (physiology, behaviour...) and on the homeostatic balance of animals prior to a selection is a good way to control the risk of trade off during the selection process, and to prevent undesirable side effects (Rauw *et al.* 1998).

A selective breeding plan ineluctably causes a loss of genetic diversity. This loss of diversity is desirable because the goal is to settle alleles associated with good production performance in the allelic pool of the farm population but it needs to be controlled. In abalone farms, a loss of diversity can be observed from the first generation of a selective breeding plan with no consequences in the inbreeding rate (Rhode *et al.* 2012; Slabbert *et al.* 2009; Evans *et al.* 2004). Nevertheless, inbreeding (result of the mating of two individuals sharing a close common ancestor leading to the rise of the homozygosis rate in the population) has been already observed in the second cycle of an abalone selective breeding plan (Rhode *et al.* 2014). This study estimated at 5% the inbreeding rate, which highlighted a major risk for the sustainability of the farm (Rhode et al. 2014).

Several negative side effects are associated to inbreeding such as increased mortality rate at the first generation of brother-sister mating in abalone (Kobayashi *et al.* 2010) and a reduced larval development after 7 cycles of oyster selection (Taris *et al.* 2006; Taris *et al.* 2007). These results demonstrate that inbreeding is clearly an important problem to solve. Inbreeding depression can be explained by a rise of homozygote locus which can lead to an expression of deleterious alleles (Lynch and Walsh, 1998). The recommendation is generally to stay at the lower rate of 1% of inbreeding (Bentsen and Olesen 2002).

For abalone, loss of genetic diversity seems to be linked with the effective population size, reproduction practices, and selection practices in only one trait (Evans *et al.* 2004). The effective population size can be defined as the actual number of genitors that will contribute to the next generation. In a farm population, the size of the broodstock pool is much less important than the broodstock observed in the wild population, due to the lost of panmixia. This reduction in effective population size can be intensified by the reproductive strategy of abalone. The high fecundity linked to the weight of females generates unequal representation of each genitor (Bilbao *et al.* 2012; Rhode *et al.* 2012). In addition, the settlement rate of larvae in France Haliotis, highly dependent of climatic conditions, contributes to increase this reduction (Personal observation).

An accurate view of the farming system where the selective breeding plan will be implemented is necessary to propose an efficient and sustainable breeding program. The unintentional selection of culling practice in French oyster hatchery is a good example of that necessity because the farm practices can significantly impact the genetic diversity in the farm (Taris *et al.* 2007). Even if the proposal of a selective breeding program is not part of this traineeship, it's important to understand the context of the farm in order to implement later an efficient program.

# **F.** Understanding of trait relationships crucial for the design of a massal selective breeding plan

France Haliotis is a small farm controlling the entire production chain. However, the small scale of the company does not allow the application of a familial selection, too costly (Table 2). The massal selection, even if it represents a risk of a loss of diversity, is scale-adapted to this farm. France Haliotis manager wants to improve some traits and control others in order to generate stable profits at middle term (Table 1).

Three observations have to be taken into account to reach this objective:

- 1- Each trait can be improved one by one. Nevertheless, because abalone cycle is long, this strategy will not be profitable on the medium term. In addition, unidirectional selection (selection based on one trait) has been shown to be unsustainable for many productions due to trade-off issues (Ferret et al. 2011; Fleming *et al.* 2011, Rauw *et al.* 1998). In *Haliotis tuberculata*, phenotypic and genotype traits relationships are unknown and need to be studied.
- 2- The massal selective plan cost is determined by the number of characters studied. Considering the high progress of genotyping technologies, phenotypic assessments are becoming the limiting process in the study of genetic and phenotypic relationships (Monget *et al.* 2009).
- 3- The massal selection will be slow with a production cycle of 3-4 years before selling. At 3 years of age, selected adults can be involved as broodstock, even if gamete contribution will be low. The growth is the most important trait for improving profit. Considering work on other abalone species, the heritability of a massal selective plan can be interesting for the farm (0.2 0.4). However the sea growing system of France Haliotis induces important variability of growth due to large environmental variation (temperature, algae quality, etc...) compared to recirculating system encounter in country such as Australia and South Africa. So the length at the end of the production cycle might be poorly linked with an actual genetic potential. If interesting traits can be measure precociously in the production cycle, which need to be verified first, younger males may be use at younger age such as two years old with three years old females.

Phenotypic correlation may not be representative of a genotypic correlation because both relationships depend of the environment (Rauw *et al.* 1998; Ernandes *et al.* 2003). Nevertheless, it has been strongly recommended to acquire biological knowledge backgrounds on the phenotypic relationships previous the realization of a selective plan in order to understand, anticipate and prevent undesirable side effects of selection (Rauw et al. 1998). Considering these observations, the knowledge of several phenotypic trait relationships in different environments, characteristic of France Haliotis husbandry practices (high density, shaking and manipulation) are important to study before considering heritability, breeding plan strategy, traceability system or inbreeding management. In addition, the extensive trait relationships study may allow to find few integrative variables and concentrate later on them during phenotypage associated with selection in order to reduce cost.

#### **<u>G. Main objectives of the study</u>**

In summary, The principal objective of this work was to study the existence of relationships between primary economically important traits such as growth and mortality and behavioural and physiological traits in different farm situation, in order to identify suitable selection traits. This objective is strongly dependent of knowledge of the biology and the homeostasis balance of abalone. It should help to understand, anticipate and prevent undesirable side effects of selection (Rauw *et al.* 1998). To answer to this problematic, a classic scientific development will be followed.

The experiment was repeated in winter and summer. However, only winter results are presented in this report.

# **MATERIAL AND METHODS**

Experiment was performed at France Haliotis facilities (48°36'46N; 4°33'30W) in Plouguerneau, France, between December 2013 and August 2014. Behavioural and physiological measures were performed in experimental facilities on-land, in France Haliotis. Growth performance measures were realized in a sea-cage structure situated on Aber Wrac'h concession between March 2014 to the end of June 2014. At the end of April, after 2 months of sea-rearing, growth and gonad development were measured before to return the abalone in the sea-cage structure. Abalone were brought back to the farm again at the end of June, and a second behavioral and physiological screening was realized as well as growth, gonad development and meat yield measures (Figure 6). The results of the summer experiment will not be presented in this report.



Figure 6: Global experimental organization, the part in gray will not be presented in this study (VGI= Visual gonad index)

## <u>A Materials</u>

#### A.1. Animals

Farmed adult *Haliotis tuberculata* (65-70 mm total shell length, 3.5 years old), bred from a single cohort from wild and farmed genitors were randomly harvested from 9 seabased breeding structure, removing them gently from the hide and placing them in bags containing circular black plastic oyster seed collectors (diameter: 140 mm). Wild adult *Haliotis tuberculata* (65-75 mm total shell length, age unknown) were collected the same day by a diver in the same area as the sea-based rearing structures. Farmed and wild abalones were transported to the land-based laboratory in a 100 L seawater tank.

#### A.2. Laboratory experimental apparatus

The experimental tanks consisted of 4 grey flat subsquare epoxy painted fibreglass tanks ( $1m10 \times 1m10 \times 0.20m$ , water volume = 100 L) with rough plastic strips on the edges to avoid escapes (Figure 7). Each tank received 75 L.h-1 of 3 µm mechanically filtered seawater and an oxygenation system was placed in each tank. Ten pairs of collectors were uniformly distributed along the tank edges to be used as hiding places. Four plastic feeders (7cm x 7cm x 3cm) containing 4 different algae (*Palmaria palmata* (Rodophyta), *Ulva lactuta* (Chlorophyta), *Saccharina latissima* and *Laminaria digitata* (Phaeophyta)) were fixed onto

the center floor at equal distance of the ten hiding places to ensure equal access of each algal species (Figure 7). The light dark regime was adjusted to season rhythm 10:14h (Light: 8h30 to 18h30; Dark: 18h30 to 8h30). To avoid stressful conditions during light changes, 30 minutes dawn (8h00 to 8h30) and dusk (18h30 to 19h00) were monitored using a dimmer (Gold Star, Besser Elektronik, Italia). Tanks were cleaned twice a week using a hose and general filters were changed every day. All tanks were continuously videotaped with six digital cameras (TS-WD6001HPSC, Sygonix Gmbh, Germany), linked to a 24h recording device (TVVR 40021, Abus, Germany).



Figure 7: Experimental tank used to study abalone behavior and physiology

#### A3. Sea-growing structure

Allibert sea-growing structure was composed of 4 square compartments (1m\*1m\*0.5m) designed for the rearing of abalone. Each quarter contained 10 rows of 42 black plastic collectors (Figure 8). Abalone were all fed once a month with *Laminaria digitata* commonly used in the farm and distributed *ad libitum* in the compartment.



Figure 8: Picture of an Alibert structure used as sea-growing structure in France Haliotis

## **B.** Stress procedure

## **B.1.** Tank repartition

Two tanks contained farmed abalone and two tanks contained wild abalone per repetition. Once in the laboratory, 10 abalones were randomly assigned per experimental tank. The experiment was repeated 3 times (n=60 wild abalone, n=60 farmed abalone in total).

After gently detaching the abalone with a spatula from the collectors used for transport, length and weight was measured and a reflective tag and number were glued to each individual (Figure 9) with cyanoacrylate glue before placing them in the tank.



Figure 9: Reflective tags glued on the ten abalones assigned to each experimental tank.

#### **B.2. Stress application**

In the laboratory, different types of stressors were applied to abalone, simulating stressors experienced during husbandry procedures (Figure 10).

- Habituation period (Day 1 to day 7) which correspond to the recovery period to mild stress due to transport and environment changes. Abalone remained 7 days without any manipulation. Previous observation have shown that the shipping stress and the tagging stress have no impacts after 3 days (Roussel pers. Comm., Hoopper et al. 2001, Cenni *et al.* 2009)
- Over-density period (Day 8 to day 14): 52 additional abalone were added in order to induce an over-density stress. The density of 52 abalone / m<sup>2</sup> is higher than the 35 abalone / m<sup>2</sup> density observed in sea-structures.
- 3) Post-acute stress period (Day 15 to day 21): Abalone were detached and shacked for 20 min day 15. They were let one hour in emersion before to be put back to their tank. During this time, the 52 non-experimental abalone were removed. After the stress, the abalone were let undisturbed until d21.



Figure 10: Experimental procedure for one repetition

# **<u>C- Measures</u>**

The variables used for the mutivariate analysis are in bold italic for the ACM analysis and bold normal for the ACP analysis.

#### C.1. Behavioral measurements

#### C.1.1. Circadian rhythm

The behavior was continuously recorded during 48h the 5th and  $6^{th}$  day of the habituation period. These measures are considered as an indicator of basal circadian rhythm. This have allowed to measure:

- The total number of movements per day (Movement of at least above 10 cm were recorded) during 24 hours of observation (**Nbre.M.D**),
- The total duration of movement per day (**Latmv**) The global activity of an abalone. Moving abalone (coded 1 if they moved more than half an hour per day or resting abalone (coded 0 if they moved less than half an hour per day (*Activ*.).

#### **C1.2 Feeding preferences**

Unfortunately almost no feeding behaviour where observed during the two days of circadian analysis. Thus it was not included in the analysis.

#### C1.3. Reactivity test:

The reactivity test was performed the last day of each period between 16:30 and 19:00. It consisted in touching gently the abalone along the foot border and turning clockwise around in 10 sec. Measurements were performed during 3 periods:

- Before touching the retraction of the foot at rest was observed (*RecAV*) ( criteria: O if relaxed, N if retracted, P in intermediate situation were imputed) (Figure 11 a,b.
- While touching the abalone, the retraction of the foot was measured (Mvt)(criteria : Y if retraction of the foot, N if no retraction, P in intermediate position were imputed).
- During the minute after the touching, the latency between the retraction of the epipod and the return to initial epipod position was measured (Latepi)



Figure 11: Pictures of an abalone in two different positions in picture a) the abalone is curled in the picture b) with I) the epipodes free II) the mantle III) the eyes.

#### C.1.4. Turn over test:

This test was performed the last day of the laboratory period, just after the haemolymph sampling procedure (see below). Abalone were gently placed on the back in the centre of a white 12-L bucket containing 5 L of sea water at the same temperature than the tanks. Video-recording was done during 4 min with a camcorder (Sony, HDR-XR155). The analyses were done with the software VLC mediaplayer. The following variables were measured (Figure 12)

- The success in a turn over test in the 4 minutes (*Success*) (Notation: 1 if the abalone turned over in the 4 minutes and 0 if there did not succeed in returning).
  - (Figure 12)
- The latency of a full turn over (Latsuccess) from the moment the abalone was placed in the bucket to the full return (Figure 12)





#### **C.2.** Physiological measurements

#### C.2.1. Physiological sampling procedure

Haemolymph was collected on the  $21^{\text{th}}$  day before returning abalone to sea-growing structure. Haemolymph (about 0.2 ml per individual) was collected from the pedal sinus using 1 ml syringe and fine needle. It was transferred into a vial and kept on ice. Samples were treated just after collection to avoid aggregation. Haemolymph were used for determining: (1) total haemocyte count (50 µl) added to 6% formaline (50 µl) kept at 4°C before analysis; (2) phagocytosis (50 µl) following the protocol of Duchemin *et al.* (2008); (3) glucose (100 µl) frozen at -20°C before analysis

Morphological and functional analyses were performed on a FACS-Calibur flow cytometer (Becton Dickinson, France) equipped with a 488 nm laser. Cells were counted for 30 s. The immunity parameters were calculated from WinMDI software.

#### C.2.2. Total haemocyte count and mortality

Before analysis with the cytometer, samples were diluted into 200  $\mu$ l of filtered and sterile seawater (FSSW) and incubated for 30 min with SYBR green fluorescent dye (Molecular Probes, 10-3 dilution of the commercial stock solution). Results were expressed as number of cells/mL-1 (**Celltot**). Haemocyte mortalities were presented by giving the percentage of dead cells present in each sample.

#### C.2.3. Phagocytosis

The phagocytosis protocol is adapted from Travers et al (2008). In summary, 50  $\mu$ l of haemolymph were deposited into a 24-well plate containing 50  $\mu$ l of sterile seawater. Haemocytes were allowed to adhere for 15 min at 18°C. Fluorescent beads (gluoresbrite YG Microspheres 2.00 mm, Plysciences, 1:100 in distilled water) were added. After 2 h at 18°C, supernatants were removed and 100  $\mu$ l of trypsin (2.5 mg.mL-1 in AASH) were added to detach the adherent cells. Plates were shacked for 10 min. Then 100  $\mu$ l of 6% formaline were used to stop the reaction. The phagocytosis index is defined as the percentage of haemocytes that has engulfed 3 or more beads (**Phago**).

## **C.3.** Production performances

#### C.3.1. Growth

Weights (W) and length (L) were measured two times: at the beginning of the experiment (T0, **Tinitial**) and in May after 2 to 3 months of rearing with low density condition (T3). The following variables were calculated:

- The weight growth per month in g/month (**X3Mdifweight.M**) was calculated as: WG=(WT3-WT0)/(NbD\*30)
- The Length growth per month in mm/month (X3Mdifflenght.M) as: LG=(LT3-LT0)/(NbD\*30)

Where:

- WT0 and LT0 are respectively the weight and the length at the beginning of the experiment
- WT3 and LT3 are respectively the weight and the length after two or three months in the sea-cage structure
- NbD is the number of days in the sea-cage structure

#### C.3.2. Gonadic development

The **Visual gonad index** (**ICgonad**) was measured in May based on Uki *et al.* (1984) with some adaptation (Table 3). It was observed on the left size of the abalone at the middle of the anther posterior axe and at the back in the middle of the bilateral axes (Figure 13).

Value of VGI	Citerions description
0	No gonad can be observed on the digestive gland. The sex of the
	abalone can not be determined
1	Gonad is observable but is flat
2	Gonad is inflated and reach the level of the shell
3	Gonad is developed above the level of the shell
4	Gonad is observable without removing the foot of the abalone

#### Table 3: Visual gonad index measured on the left side



Gonadic development in the midle of the bilateral axes (=3 in our scale) On the back of the abalone.

Figure 13: Gonad of a mature abalone

#### C.3.3. Mortalities and diseases

The mortality was recorded during the laboratory period and during the sea-growing period before T3 growth measurement (*Deadmay*, 0=alive, 1=Dead). The presence of mantle abscess on the gonad was also recorded (*Abscess*, 0=healthy 1=Abscess).

# C.4 Statistical analysis

## C.4.1 Materiel

Access and Excel programs (Microsoft Office 2010) were used to format the data. Statistical analysis were performed with R 3.0.3 software. Rcmdr package was used to test the normality of the residuals and the homogeneity of variances. When these conditions weren't verified a non parametric kruskal wallis test were realized. Factominer packages were used for multivariate analysis. Variables were center and scaled before the analysis. The basic functions of Factominer were used to draw ellipses of confidence around the gravity center of population for each factor level.

## C.4.2 Procedures

## First step: Description of the dataset

Each individual was described by a set of 17 variables. This step allowed to understand abalone responses to the different test and situation. The average and the variability of these variables were presented.

#### Second step: Multivariate analysis after imputation

In order to represent the relation between the variables, two different multivariate analysis were conducted. Firstly a principal component analysis (PCA) was performed on quantitative variables (in bold in the material and methods) in order to explore the structure of our quantitative multivariate dataset. A multi-factorial component analysis MCA was used with qualitative data (in bold and italic in the material and methods) in order to represent the relation between factors and factors level of qualitative variable. To perform this analysis, and to relate qualitative variable to the growth, growth variables were transformed in qualitative variable with three modalities: (0: individuals in the first growth quartile, 1: individual

between the first and the third quartile of growth, 2: individuals with the highest growth quartile). In order to facilitate the reading of the MCA output the two growth variable **X3Mdiflenght.M** and **X3Mdifweight.M** were renamed by *G.L* and *G.W* posterior to their factor transformation. *G.L\_2* and *G.W\_2* were the two best growth categories.

However, multivariate analysis requires a complete data series. Due to mortality before T3 growth measurement, missing data during haemolymph sampling or video analysis with abalone hidden during recording, some series were incomplete. In order to increase the degree of freedom and use the information of individuals with few missing variables, a regularized iterative imputation was performed (Josse et al. 2012). It's important to note that iterative imputation can create false correlation in the dataset (Quinn and Mc Keough 2002). To reduce this risk, a recommended methodology was followed (Quinn and Mc Keough 2002): the multivariate outlier (4 abalone) and individuals with more of 50% of missing data were deleted (19 individuals dead during the first laboratory period). A data set of 97 individuals was obtained, reducing the rate of missing data to 13%. The package missMDA was used to perform the imputation. This imputation is realized after an optimization step of noise reduction, in order to implement value by iteration independently of the noise and decrease the risk to create false correlations. This had allowed to obtain a imputed complete data set with 97 individuals. This imputed dataset were only used for the PCA and MCA representation.

#### Third step: Quantitative assessment of the correlations suggested in the explorative step and analysis of the effect of trait levels on the growth

The power of the relationships previously found in the imputed dataset were tested and quantified in the dataset using linear models. The effect of some factors level on the growth to determined trait level which included a higher growth in our population was also tested by ANOVA.

Prior to the ANOVA test, the quantitative variables correlated with the growth were transformed in qualitative variables with three levels of factors as we already explained for the growth (in the second step).

ANOVA analysis was performed on some quantitative data transformed in qualitative data using quartile or 10% upper or lower values.

Behavior and physiology of abalone with the best growth (upper quartile) were compared to abalone of the lowest growth (lower quartile).

# **RESULTS**

## A. Abalone trait description

In this population 6 qualitative traits and 11 quantitative traits were measured (Table 4; Table 5).

#### A.1. Mortality and growth

A mortality of 44% was observed between T0 and T3 (Table 4). The mortality occurred for 15% during the 3 weeks of the laboratory period and at 85% between the end of the laboratory period and the growth measurement in May. At that time, 6% of the abalone presented abscess on the gonad (Table 4).

Both growths in length and in weight were really low during the three months of rearing and the 75% of the population have lost weight in May and the average growth in length was 0.23 mm/month (Table 5). An important variation coefficient was observed for these two variables indicating a large individual variability (Table 5).

#### A.2. Physiology

The total haemocyte count was recorded for 60 % of the population and showed an important individual variability whereas less variability was observed for the phagocytosis efficiency. In May, 25% of the population were already ripe (VGI>2) and the other 75% of abalone started (VGI>2) to generate gonad tissue (Table 5).

#### A.3 Behavioral test

In the turn over test, more than 84% of the abalone succeeded in returning in less than 4 minutes (Table 4). The latency of success presented an high variability (Table 5).

During the circadian rhythm measures, two groups of abalone were identified: 49% of the abalone did not move or moved less than half hour per day of observation, while 51% of the abalone made in average 3 movements per day of one hour in average (Table 5).

<u>Table 4 Responses of 120 abalone observed during different laboratory test situations</u> (habituation, high-density, post-acute stress period) and in the sea-growing structures (basic <u>dataset analysis, 6qualitative variables</u>)

Variables	Yes	No
<b>DeadMay</b> (The abalone was died in	43.8%	52.2%
may)		
Absces (The abalone presented an	6%	94%
abscess on the foot in may)		
Success (The abalone succed in the	84%	16%
turn over test)		

<b>RecAV</b> (The abalone had the foot	58%	42%
retracted before the toutching)		
Mvt (The abalone had made a	22%	78%
retraction mouvment during the		
toutching)		
Activ (The abalone had moved more	49%	51%
than half an hour per day)		

# <u>Table 5 Responses of 120 abalone observed during different laboratory test situations</u> (habituation, high-density, post-acute stress period) and in the sea-growing structures (basic dataset analysis, quantitative variables)

<u>Variable</u>	Average	<u>Variation</u> coefficient	<u>Q25%</u>	<u>Mediane</u>	<u>Q75%</u>
WT0g(weight at TO)	49.11	0.28	40.38	50.11	57.12
Tinitial mm (lenght t To)	68.98	0.01	65.44	69.35	73
X3Mdifweight.M g/month(growth in eight per month)	-0.86	1.61	-1.68	-0.62	0
X3Mdiflenght.M mm/month (growth in lenght per month)	0.23	1.95	0	0.25	0.46
ICgonad (Visual gonad index in may)	1.55	0.35	1.25	1.5	2
Phago % active cells(% of heamocytes active in the phagocytic process)	73.5	0.14	69.80	75.71	92.06
Celltot nbrCells/sample (Number of cells per sample)	3633	1.08	1281	2134	4786
Latsuccess min:ss (Latency of success to make a full turn over)	1 :10	0.67	00:42	00 :54	01:20
Nbr.M.D number of movement/day	1.75	1.1	0	1	3
Latm Average total duration of mouvment h/day	0.61	1.0	0	0.48	0.92
LATEPI s	39	0.54	19	37	60

#### **B** Relationship between growth behaviour and physiology (n=97)

#### **B.1.** Principal component analysis (PCA) on the quantitative variables

The PCA on the 11 quantitative screening variables showed that the first component explained 19.7% and the second component 17.1% of the total variance. The most important loadings on the first component were the average total duration of a movement per day (Latmv,.73), the growth in weight (X3Mdifweight.M, 0.63) and the initial size (Tinitial, 0.55). This component represents the activity and the growth score (Figure 14, a). For the second axe the most important loadings were the number of cells (Celltot, 0.75), the visual gonad index (ICGonad, 0.64), the latency of success in the turn over test (LatSucces, 0.58). This component represents the abalone physiologic condition score (Figure 14 a).



Figure 14: PCA representation of the growth, behavioural and physiological variables measured during different laboratory tests and sea-growing situation (imputed data, 10 quantitative variables, a) variable, b) individuals)

No cluster differentiating wild and farmed abalone or died or alive abalone were observed based on PCA analysis. (Figure 15).



Figure 15: Individual distribution of a) abalone dead and alive in May (respectively <u>0</u> and <u>1</u>) b) wild and farm abalone (respectively S and E) obtained with PCA analysis

#### **B.2.** Multiple component analysis (MCA) on the qualitative variables n=108

The MCA on the 9 qualitative variables showed that the first component explained 17.3% and the second component 13.8% of the total variance. The most important loadings on the first component were the origin of the abalone (*Origin*, 0.45) and the mortality (*deadmay*, 0.5) as well as the length growth (*GL*, 0.39). This component represents the production variables as well as the origin of the abalone (Figure 16, a). The factor level relationships plot showed that the farm abalone (*E*) were related less mortality (*deadmay\_0*) and a better growth in length (*GL\_2*) (Figure 16, b).

For the second component, the most important loadings were the position of the feet before touching the abalone during the reactivity test (*Recav*, 0.55) and the retraction movement when they were touched during the reactivity test (*Mvt*, 0.55). This component represents the position at rest and the reaction after a contact stress situation (Figure 16, a). The factor level relationships plot showed that abalone which were relaxed before the reactivity test (*Recav\_O*) made more protection movement (*Mvt-O*) (Figure 16, b).

The factor level relationships plot showed also that the growth in weight ( $G.W_2$ ), the category of mouving and relax abalone ( $Mouve_1$ ), ( $Mvt_0$ ), ( $Recav_N$ ) are related (Figure 16, b). The abalone which have moved and which were relaxed are more likely to be in the best weight growth category. The lower categories of growth in weight and in length ( $G.L_0$ ,  $G.W_0$ ) were closer to the presences of abscess ( $Abesces_1$ ), The best growth in length category ( $G.L_2$ ) seem to be linked uniquely with the Farm origin (E) (Figure 16, b).



**Figure** 16: MCA representation of the growth, behavioural and physiological variables measured during during different laboratory tests and sea-growing situation (imputed data, qualitative variables a) Factors relationship b) Factors level relationship)

## C Analytic analysis in the basic data set, and trait level impact (n=97)

#### C.1. Quantification assessment of the relationships by linear regression

Linear relationships were found between growth in weight, the average total duration of movement per day (P =0.016), the gonad development (P=0.003), and the phagocytosis rate (P=0.007) and between the growth in length and the latency of success in the turn over test (P<0.001).

#### C.2. Study of the impact of some factors level on the growth

The 10% abalone with the highest latency before a full return in the turn-over test had a lower growth compared to the other categories (pvalue=0.009) (Figure 17).



Figure 17: Latency of turn over (0 : 10% quickest to return, 1 : intermediate, 2 : slower to turn over) effect on the growth in lenght of abalone submitted to a turn-over test

Comparisons of the abalone in the best growth quartile to the abalone in the lowest growth quartile showed that nearly non differences were observed for behavioral and physiological variables. However, abalone from the best quartile of the total duration of movements per day tend to have a better growth (p=0.02) compared to abalone from the lowest growth quartile (Figure 18). The abalone from the lowest quartile of visual gonad index have a lower growth in weight compare to the two other groups (p = 0.031) (Figure 18).



Figure 18: A) Total duration of mouvment per day (0: 25% of abalone which have smallest duration of mouvment per day, 1: intermediate, 2: 25% of abalone which the highest duration of mouvment per day) B) ICgonad (0: 25% of abalone which have the lowest visual gonad index, 1: intermediate, 2: 25% of abalone which have the highest visual gonad index).

# Discussion

The aim of this study was to understand how economically important traits such as growth and mortality were related to behavioural and physiological traits. To achieve this objective, 120 animals were placed under stressful situations in laboratory and usual rearing conditions. In total, 17 variables were measured.

# A. <u>Growth performances and survival linked with behavioral and</u> <u>physiological traits</u>

This study showed that growth is positively related with the average duration of movement per day in laboratory condition, gonad development and negatively related with the phagocytosis efficiency at the end of the 3-week stress application. The abalone with the best weight growth were the ones with wanderer behaviour during the 24-h activity rhythm, and a foot relax in the reactivity test. The growth in length was also positively related with the total haemocyte count and negatively with the latency of return success during a turn over test. In addition, the study showed that the mortality was related to a fail to return in the turn over test at the end of the three weeks of stress condition.

A good weight gain associated to a better gonad development has already being observed in the literature (Bilbao *et al.* 2012; Roussel *et al.* 2013). Indeed, abalone larger than 50 mm with a good growth are more likely to be in good physiological condition and to produce gonad when the water become warmer (Bilbao *et al.* 2012).

The relationship between growth in sea structure and circadian rhythm has never been tested for abalone. The results showed that wanderer animals, being either more motivated for moving, might access more to the food in a cage and thus had a better growth in contrast to sedentary abalone. Another hypothesis is abalone highly motivated for food moved more to get this food. These two strategies of movement are in accordance with Cenni *et al.* (2010) who showed that in a population of *Haliotis tuberculata* each individual, independently of its sex, adopts one of two locomotion strategies, behaving as either a "wanderer" or a "sedentary". Even if locomotion represents an important metabolic cost (Davies and Hawkins 1998), keeping oneself adherent to the substratum seems to be more costly (Donovan and Carefoot 1997). This could also explain the link observed between a relax behaviour in the reactivity test and the best growth in weight categories in the multi component analysis.

The growth was also positively related with the total haemocyte count and negatively with the latency of success. The relationships observed between the total haemocyte count and the growth in length might be explained by its metabolism multi-function in abalone. Nevertheless this result needs to be taken with caution because it seems to be sensitive to outliers. It's also difficult to understand the biological sense of the measurement after 3 weeks of measurement considering that total haemocyte count is sensitive variable to farm stressors such as shaking (Malham *et al.* 2008). It might represent stress response more than metabolism performance of abalone in this situation.

In this study, abalone were highly manipulated during the screening. A negative relationship between the growth and the phagocytosis efficiency was observed while no relationship with survival was observed. An acute stress has been shown to induce a decrease of the phagocytosis efficiency 1h after stress application (respectively Malham et al. 2008, Cheng et al. 2004, Hopper et al. 2011). These reduced phagocytocis efficiency were related with higher mortality and considered by these authors to be an indication of immune depression following to a stress. However, in our experiment, measurements were performed 1 week after the acute stress application in contrast to Malham et al. (2008) and Cheng et al. (2004) experiments where sampling were performed maximum 48h after stress application. This delay could explain some of the differences observed with the literature. Anyway, the relation between the growth and the reduced phagocytosis efficiency need to be followed during the selective breeding plan. Indeed if these phenotypic correlations are linked with a genetic correlation, it could generate trade off with important consequences for the production performance. If the lower phagocytosis efficiency is representative to a lower ability to resist to pathogen. (Hopper et al. 2007, 2011, 2014; Travers et al., 2007, 2008), animals with the best growth are more likely to die during mortalities picks observed during summer vibrio outbreak, as it is already observed in farm conditions (Huchette pers. com.).

Abalone that were retracted and who couldn't return during the turn over test after the 3 weeks of stress were more likely to die compared to abalone quick to return who presented a better growth. In the literature, the turn over test has been shown to be a good indicator to measure the energy reserves of an abalone (Baldwin *et al.* 2007). After three weeks of stress, abalone quick to turn over might be the ones with the most important reserve and resistant to stressors encountered during the experiment. These abalone were able to resist to pathogen during the rearing period in the sea cages.

#### B. Screening profile consequences for the selection

France Haliotis company is interested in starting a massal selective breeding plan. In order to reduce the loss of genetic diversity and limit the risks of trade-off apparition, it's interesting to select abalone broodstock on several traits. A pertinent trait in a selective small farm breeding plan is a trait linked with a better productivity, easy and cheap to measure, repeatable in the year, non invasive to measure, heritable and not genetically linked with other deleterious trait. In abalone production, the low growth is identified to be the most important trait to improve. The growth in length and weight are already well described in the literature (Gerber *et al.* 2012).

Nevertheless, one of the objectives of this experiment was to find traits related to a good efficiency in the farm to select and/or to control during the selection process in order to anticipate trade off negative side effects.

A synthetic table of advantages and disadvantages of some identified traits are presented below according to the criteria explained above (Table 6).

Trait	Advantages	Disadvantages	Recommendation
Latency of	Easy, quick, and cheap	Dependant of the	Critera to select
return in a	to measure	temperature	
turn over test	Good indicator of		
	metabolism and		
	energy reserves		
Average total	Good relationships	Time-consuming and need	Critera to select
duration of	with growth	of video equipment	
movement	Good indicator of		
per day	growth performance	Dependant of the	
		temperature	
Gonad	Easy and quick to	Risk of trade-off because	Control during the
development	measure	an higher gonadic	selection
		development enhance	
	Good relationships	mortalities (Roussel et al.	
	with growth	2011)	
Phagocytosis	Inverse relationship	Risk of trade off because	Control during the
efficiency	with growth	the phagocytosis	selection
-	_	efficiency is a good	
		measurement of the	
		immune system activity	
		(Hooper et al. 2007)	
		Potentially invasive to	
		measure	

Table 6: Advantage and disadvantage of traits related to growth for the selection

Based on the relationships shown in this study, a recommendation would be to complement selection based on weight gain, growth in length and meat yield of 3.5 year old abalone with a turn over test and to study 24-h rhythm. The 24-h rhythm study could be complemented with a feeding behaviour records by placing broodstock in tank during one week with shelter and feeders in order to select wanderer animals. A set-up based on Werner *et al.* (1995) experiment could be used where animals would be placed in a shelter with no food and would have the opportunity to move across an open area to reach another shelter with food.

Another trait to consider in selection would be a reduced gonad development for abalone used in sea-structure rearing. If the phenotypic relationship find between growth and

gonadic development is explained by a genetic correlation, a good growth performance selection might result in abalone with higher gonad development and potentially could enhance a higher mortality rate (Huchette pers. com.). In this case, if a co-selection with a reduced gonad development and improved growth is built, it should be expected slower selection progress. It will be important to study the genetic correlation between these two traits because the two correlations could have opposite sense (Ernandes *et al.* 2003).

Considering that the gametes from the males are produced in excess and are nonlimiting factor to reproduction compared to female gamete number, a way to reduce cycle selection time would be to mate males of two year old with females of three year old (Haffray pers. com.). A limitation of this strategy is the majority of the weight gain is realized during the third year and the genetic potential of growth in the two first years of rearing is not necessarily related with the growth performance in the third year (Boudry pers. com.). Also, the heritability observed during the second year might not be well correlated to the performance observed at commercial size (Ernandes *et al.* 2003). Therefore to use our results in order to assist the selection on animals of two years old, complementary analysis need to be done. They could be really usefull for the implementation of a faster selection.

#### C. <u>Wild and farm differences : implication for reseeding practices</u>

In this study, differences between the wild and the farmed population were observed. The wild abalone seemed to suffer more of the high-density and handling stressors than farmed abalone with reduced growth in size, higher mortality rate, and less success in the turn over test.

France Haliotis reproduction strategy in 2013 consisted in mating wild abalone from Brittany origin with the biggest farm abalone of the 2013 generation obtained from the seastructures. It can be assumed that genetic diversity differences between farmed abalone of the study and abalone caught in the wild is probably reduced because the reproduction strategy of France Haliotis used an important proportion of wild genitors. The differences of mortality, growth and behaviour probably result probably more from a different ontogeny environment than of genetic differences (for example due to inbreeding). Wild abalone were submitted to natural selection pressure while farmed abalone experienced indirect selection pressure related with the rearing system (Figure 5).

Considering that farmed animals had a better growth in length and weight gain, the relationship between growth and movement might be one of the first observable consequences of the domestication process. Indeed, it was already found that animals selected for better growth moved more than unselected animals (Robinson *et al.* 2013). Another hypothesis would be that farmed abalone, which had a better growth in the experiment, were used to farm structures because they grew in these structures during more than 3 years. As a consequence, they were less stressed and moved more to get the food.

If the growth environment during the younger stage of the abalone life has consequences on their behaviour, it will be important to study the consequences of rearing during juvenile stages before reseeding practices. The company will have to apply a different selective breeding plan with more genetic diversity (i.e. use more wild genitors) as well as probably adapt husbandry rearing to have good results with reseeding programs.

#### D. Limits and interesting complements of the previous screening

The high mortality rate (43%) observed after 3 months of experiment is an important limitation of the study. This mortality might create a bias in the study of the relationships between growth and the others factors. Growth was only measured for animals able to survive to the 3 weeks of stress applied in winter. However, this mortality is often met during farm practices, with mortality up to 40% after a manipulation during the summer period (Huchette, pers. com.). Abalone still remains a sensitive species to farm stressors (Travers *et al.* 2008) and indicate that in Europe, the *H. tuberculata* farming is still in the very early stage of the domestication process.

In relation to this high mortality, a loss of weight was still observed 3 months after the laboratory experiment, showing that stressors experienced during the experiment can have long term consequences on growth. Similar phenomena are observed on farm and induced a modification a farm procedures in France Haliotis. Indeed, since 3 or 4 years, the farm manager reduced handling during rearing in order to reduce these stress side-effects. However, it's very important to integrate the abalone response to stressors because animal have to face these stressors regularly during their rearing (Figure 5). The two growth variables measured in the experiment after 3 months were more related with the individual capacity to resist to the chronic and the accurate stressors than to agronomic efficiency in non-stressful situation. A second growth measurement was realized in July after 6 months of rearing and will give complementary indications. An interesting complement to validate the outputs of this study would be to measure and tag abalone with gently handling, let them in sea-cages during 1 year (summer and winter season) and to test their 24-h activity one year later.

A third limitation of the study was the repetition effect. Its impact on the structure of the data set will have to be verified more precisely. This effect is probably due to the change of sea-temperature during the experiment. The first repetition was performed at 9 degree average sea temperature while the last repetition was performed at 11 degrees. This probably explains why total movement duration was higher for the third repetition compared to the first repetition. Indeed, abalone are poikilotherm and their metabolism activity depends of the temperatures of his environment.

The movement rate and feeding pattern was low considering other experiments realized in France Haliotis at the same period in 2012 (Roussel pers. data.). This can be explained by the fact that the temperature was really low for the two first repetitions (9°C) compared to the 11 degrees average temperature observed in 2012. *Haliotis tuberculata* is

known to be a nocturne animal. However no difference of movement was observed between day and night time during the 24-h circadian analysis in contrast to Cenni *et al.* (2010). This could be explained by the fact that the observations were done in winter (Roussel, 2013). . The experiment will need to be repeated in summer in order to have a more complete view of the different variables relationships.

# Conclusion

In this study, it was found that some behavioral traits (duration of movement during a 24-h rhythm observation, latency of return in a turn over test) and physiological traits (phagocytosis efficiency, visual gonad index) are linked with the growth and mortality after a stress period in our population. This study showed that behavioural tests could be interesting tools to evaluate the ability of an abalone to support the rearing condition and realized a good growth even under stress conditions. It also showed that; even if the abalone production is still at the very beginning of the domestication process, wild and farmed abalone already have different behavior and growth. The environment of the abalone growth in his early development stage may have an impact on it.

The second step will be to build a sustainable selective breeding plan in France Haliotis, to measure the heritability of the interesting production traits and to study the genetic relationships of these traits. Assuming that behavioral and physiological traits are heritable, this study could have three important outputs for the selective breeding plan implementation:

- The possibility to use behavioral and physiological traits in the selection in order to work on larger trait diversity and/or increase selection efficiency
- The potential existence of trade off with the phagocytosis efficiency and gonadic development will need to be controlled during the different production cycle
- The potential use of behavioral tests on young abalone will need to be tested and might be interesting to inform on their growth genetic potential

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Titre français : Etude de relations entre croissance, physiologie et comportement de l'ormeau européen *Halio Tuberculata* dans la perspective de la mise en place d'un plan de sélection

Titre anglais : Relationships between growth, physiological and behavioural traits of the European abalone *Haliotis tuberculata* in the perspective of a selective breeding plan implementation.

**Résumé :** Après dix ans de production, France Haliotis, une ferme halioticole située à Plouguerneau en Bretagne, a le projet de mettre en place un plan de sélection dont l'envergure soit adaptée à la taille de l'entreprise. La croissance et la mortalité sont les deux clefs pour augmenter la rentabilité de la production. Avant de mettre en place le plan de sélection, une description du phénotype de l'ormeau à partir de variables de croissance, comportementales et physiologiques a été réalisée. L'objectif était d'étudier les relations phénotypiques entre ces variables, d'en sélectionner un nombre restreint parmi les plus intéressants pour la sélection et d'identifier le risque d'apparition de trade off. Cet hiver, dix-sept traits phénotypiques ont été mesurés sur 120 ormeaux lors de trois semaines d'exposition à des agents stressants en laboratoire et après 2-3 mois d'élevage en mer. Ces agents stressants étaient liés à des pratiques d'élevage (sur-densité et manipulation), Les relations entre les variables ont été étudiées avec une analyse en composante principale et une analyse en composante multiple. La croissance en mer serait reliée à la durée moyenne de mouvement par jour et le développement gonadique. Une relation inverse a été observée avec la latence de retournement et l'efficacité phagocytaire. Si ces traits sont héritables, des tests comportementaux pourront être utilisés pour compléter la sélection faite sur la croissance. Lors de la sélection, l'évolution du développement gonadique et de l'efficacité phagocytaire devront être suivis afin d'éviter l'apparition de trade off, potentiellement délétère pour la production.

Abstract: After 10 years of production, France Haliotis, an abalone farm located in Plouguerneau, Brittany, wants to set up a selective breeding plan. The growth and mortality were identified as the two key points to improve in order to make profit. Before to start the selective breeding plan, a phenotype description including growth, behavioural and physiological variables was performed. The aim was to study the relationships between these variables and to find the traits which could be interesting to include in breeding plan as well as the presence of trade off. Seventeen variables were measured on 120 abalone in winter during three weeks of stress exposure related to farm practices (over-density and handling) in laboratory condition and after 2-3 months of rearing in sea cages. The relationships between the variables were studied using principal component analysis and multiple component analysis. The 2-months growth in sea-structure was found to be related with the average movement duration per day during a 24h-activity observation, the gonadic development and inversely related to the latency of return in a turn over test and the phagocytosis efficiency. If these traits are heritable, behavioural tests could be used to assist the selective breeding plan and to evaluate the genetic potential of abalone. In addition, the evolution of the phagocytosis efficiency and the gonadic development need to be followed in order to prevent potential deleterious effects on the growth and on the mortality

Mots-clés : Abalone, Screening, Growth, Behoviour, Physiology, Multivariate analysis, selective breeding plan Key Words: Ormeaux, Phénotypage, Croissance, Comportement, Physiologie, Analyse multi variée, Sélection