Rapport de stage individuel

4ème année

Compressibilité et résistance à l'écrasement de la coquille de la moule commune *Mytilus edulis* : effets du temps d'immersion et du degré de parasitisme

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Presentation of the host institution

The host institution for my 4A internship is the Station Marine de Wimereux. This is a department of the University of Lille since January 1999 and it belong to the Réseau des Stations et Observatoires Marins of the CNRS. This institution has several main missions that are education, research realized by researchers and teacher-researchers, and observation of costal and littoral ecosystems. These researches are conducted as part of the Laboratoire d'Océanologie et de Géosciences – UMR LOG 8187, which associate the Centre National de le Recherche Scientifique (CNRS), University of Lille and University of the Littoral Côte d'Opale. The research developed by the LOG includes ecophysiology, physic, chemistry and biology at the interface between ocean and continent, ocean and atmosphere and water and sediments, axed on how the costal ecosystem work throw carbon flow and its associated elements, at different scales. The Station Marine de Wimereux fits with the politic of the observation of littoral and coastal environment of the Channel and the North Sea and take part in the Service d'Observation du Milieu Littoral, labelled by the Institut National des Sciences de l'Univers.

The Station Marine de Wimereux rallied researchers, teacher-researchers, doctoral students, post-doc and several trainees, as well as a technical staff including engineers and technicians. In the Station, many scientific projects are realized as the project SOLACE.

Presentation of missions

During this internship, my mission took part of the project SOLACE, i.e. Résilience de la moule commune *Mytilus edulis* aux perturbations climatiques et anthropiques. This project globally studies impact of the environmental changes induces by climate change and global warming on mussels *M. edulis* and on mussels' aggregates.

In this project, I especially studied compressibility of mussels' shells and shells crushing resistance of these mussels *Mytilus edulis*. These parameters were basically studied in comparison with immersion time and parasitism degree, to analyses effects of these parameters on shell resistance and compressibility. With the several sampling and data measurements realized, compressibility and shell strength were mainly compare to biometric parameters, mainly mass, and to immersion time with a division of the foreshore in 3 different levels : the most the level is low in the foreshore, the most is immersion time. Finally, to compare these data in different environments, two sites have been studied during this internship. The effect of parasitism on mussel shells has been studied mainly in comparison to non-parasited mussels and not between mussels with a different parasitism degree.

This mission leads to the redaction of a scientific rapport that is presented in the next parts of this work and to a compilation of data collected for the state-of-art work (Annex I and II).

Presentation of the progress of the mission

My internship lasts 12 weeks from 19th April to 9th July. The goal of this internship was to determine the relation between strength of mussels' shell and its compressibility and biometric parameters for different immersion time, i.e. localisation on the foreshore, and for parasited and non-parasited mussels. These analyses allow to specify the nature of the relationship that may exist between mussels and predator for example. During this period, my mission was separated in different parts, that correspond globally to the successive steps of scientific reflexion : these steps are presented in Table 1.

Table 1. Different steps of my internship and average time that each step took

Part of the mission	Average time
Bibliography	1 week
Field trip and test-experiment to establish and calibrate the protocol	1 week
Processing data from test-experiment to have a first idea of which results we expect	1 week
Field trips and experiments	4 weeks
Processing data in parallel of experiments	
Realization of statistical tests	2.5 weeks
Preparation, presentation and discussion about results with 3 researchers of SOLACE	0.5 week
Redaction of the rapport	2 weeks

Thanks to this division of work, my goal was reach twice because I had the time to analyse 2 sites.

During my internship, each week, I discuss about each step of my mission with my internship supervisor. These discussions were useful to help me to continue my work on the right way and to concentrate on the relevant elements of my mission.

Moreover, thanks to the different projects that are realized in the Station Marine de Wimereux, I could also participate to various field trips in relation to the project SOLACE and in relation to an ongoing survey of the presence, abondance and distribution of the invasive Asian shore crab *Hemigrapsus sanguineus* along the French shore of the eastern English Channel.

Introduction

On a global scale, coastal marine ecosystems are one of the most vital ecologically and socioeconomically. Indeed, food, raw material and services from these ecosystems represent each year more than 14 billion dollars, that represent 43% of the global economy. These ecosystems are various, regarding different parameters as mineralogic substrates, morphology and sediments. On a global scale, rocky intertidal ecosystem is considered as one of the most variable and stressful places thermally. That fact, combined to the actual global warming, leads to various changes in all parameters that define these ecosystems. These changes lead to a modification of biodiversity, abundance and geographic distribution of species. In this context, intertidal communities are ideal targets to study the effects of climate change (Laurent Seuront, personal communication, February 2021).

These effects have been largely studied in the scientific literature, mainly considering gastropods. These organisms are both impacted by parameters influenced by climate change. Gastropods are characterized by their phenotypic plasticity : a gastropod, according to its environment, will adapt to fit with parameters (Leonard, 1999). Several ecotypes can be defined. An animal allocates its energy to three mechanisms to increase its fitness : growth, reproduction and defence (Brönmark, 2012). This allocation changes with parameters of the environment : presence of predators or individuals of the same species, presence of high or low concentration of calcite. In defence, gastropods will adopt two type of strategies : avoidance behaviour or defence behaviour. This behaviour is defined mainly by shell strength face to the predator's strategy of attack and the cost of both : it is referred to as trait compensation (Rundle, 2001; Cotton, 2004). This shell strength depends on a range of parameters such as the surface regularity of the shell, its thickness, its mass that is directly correlated to this thickness, the structural homogeneity of the shell, concentration of calcite and pH (Brönmark, 2012 ; Natesan, 2016).

These characteristics are, however, also pertinent in bivalves and specifically for mussels. Thus, the state-of-the-art highlight that a rise in temperature reduces shell surface up to 50% (Mackenzie, 2014). This reduction leads to a reduction of compression forces distribution and so leads to a reduction of shell strength. In a similar manner, an augmentation of temperature and an acidification of the environment leads to a reduction in shell compressibility (Mackenzie, 2014). Shell morphology also have an impact on shell resistance to compression, according to the distribution of forces on the shell. Typically, a round-shaped shell is more resistant than a flat-shaped shell (Cotton, 2004). However, pH seems to have an effect on this morphology as an acidification of the environment leads to distal pole of the shell thicken (Mackenzie, 2014). Moreover, shell resistance does not only depend on its physico-chemical properties. Shell's resilience decreases with time, as the mechanical resistance of the Californian mussel *Mytilus californianus* decreases with time, and the augmentation of both the frequency and amplitude of compression (Crane and Denny, 2020).

In France, marine ecosystem products represent an important part of activities from a socioeconomic point of view with its 4,668 km of shoreline. These activities are especially important in the region Hauts-de-France where mussel production represents 2,600 tonnes per year and especially mussels *Mytilus edulis*. In the context of global warming, it has been noticed that its habitat is threated and that a high mortality of individuals of this species is observed. This work fits in the project SOLACE, funded by the Hauts-de-France State and the European Fund for Maritime Affairs and Fisheries, that investigates the impact of climate change on *Mytilus edulis*.

In one site that have been observed, La Pointe aux Oies, a parasite is present on mussel's shells. The state-of-the-art highlights two main type of parasitism. Their characteristics can allow to understand how this parasite works. The first type of parasitism is an infestation by endolithic cyanobacteria, that spread mainly in cold water (Ndhlovu, 2019). The second form of parasitism is by the trematode *Himasthla elongata*. This trematode gives to mussels the capacity to survive better in warm water but worse in cold water than non-contaminated mussels (Selbach, 2020). We do not have the name of the species, but as stated above it is an endolithic cyanobacteria that bores the mussel shell, as such increases its porosity and decreases its resistance to crushing (Zardi et al., 2009).

Mussel's shell is composed of calcite and aragonite. It is structured with prismatic arrangements in column of calcite with the central layer of each valve composed of aragonite. Each layer of aragonite is separated with a protein matrix. This organic content gives property of compressibility of mussel's shell that do not have gastropods' one. These components allow mussel's shell to be more resistant to tension than compression. The crushing resistance of a molluscan shell depends mainly on its middle calcareous prismatic layer. Thanks to this composition, shells can be repair if it is damaged (Mackenzie, 2014).

Thanks to this state-of-the-art work (compile in Annex I and II), and in the context of SOLACE, regarding problems of global warming and its influence of intertidal marine ecosystem and the study sites of La Pointe aux Oies and Le Portel, this work will study the shell crushing resistance and compressibility of blue mussel (*Mytilus edulis*) depending on parasitism degree and the immersion time, depending on the position on the foreshore. We expected that (i) the most a mussel is submerged, so, the most it located lower in the foreshore, the most its shell calcify and the most this shell is resistant to crush and is compressible ; (ii) the most a mussel is parasite, the least its shell is resistant to crush and compressible; and (iii) the most a mussel is submerged, the most its shell is resistant and the least it receives solar beams, so the least it is parasite.

Materials and Methods

State-of-the-art

By searching information about molluscs and their relative shell crushing resistance, articles from the data base of the Station Marine de Wimereux and from website Web of Knowledge were used to establish this review. Here are number of articles found on the platform Web of Knowledge by using different key-words (Table 2) :

Table 2. Number of results of articles found in Web of Knowledge by searching some key-words

Key-word 1	2	3	Number of results
Mussel			48194
Mussel	Crush		140
Mussel	Crush	Resistance	10
Mussel	Shell		5367
Mussel	Shell	Resistance	180
Mussel	Parasitism		234
Mussel	Parasitism	Shell	43

Study sites

In this work, 2 sites are studied : La Pointe aux Oies and Le Portel. La Pointe aux Oies is a rocky intertidal reef typical of the rocky habitats found along the French coasts of the eastern English Channel (Chapperon and Seuront, 2009; Seuront and Spilmont, 2015; Spilmont et al., 2016; Moisez and Seuront, 2018). It is exposed to the dominant SSW characterizing the area and hosts a large mussel bed where *M. edulis* abundance typically ranges between *ca.* 500 and 5,000 individuals.m^{-2} (Spilmont et al. 2016). Le Portel is an intertidal rocky reef found along the French coasts of the eastern English

Channel. The Fort de l'Heurt is a ruin located on Le Portel. Thus, the foreshore is dominated by small-scale topographic features such as crevices, pits and small boulders. It is exposed to the dominant SSW characterizing the area (Moisez and Seuront, 2018).

Figure 1. Localisation of the 2 study sites
Source : Google Maps (2015)

Sampling

For each study site, more than 100 mussels were sampled at low tide for each level on the foreshore : Low, Medium and High levels (Table 3). For La Pointe aux Oies, parasite mussels were sampling on high level.

Table 3. Number of mussels sampling for each site and for each level

	La Pointe aux Oies	Le Portel
Low level	125	110
Medium level	109	115
High level	125	115
High level, parasited mussels	227	/

To reduce the influence of environmentally-induced differences in the development of each individual, sampled mussels were specifically chosen as vertically oriented to the substrate to reduce any environmental effect that could lead to an asymmetry between valves.

To avoid differences between samples because of growth differences, 3 sampling were realized within 3 weeks : one in Le Portel and two in La Pointe aux Oies to sample non-parasited and parasited mussels.

Until their dissection, mussels were kept alive in aquaria filled with running aerated natural seawater.

Manipulation

Dissection

Measurements were performed on fresh mussels' shells (Figure 2). To avoid introducing a bias related to the level of dryness of mussels, these steps were standardized as described hereafter. Thus, the first step, dissection spends 40 minutes. The dissection consists of opening mussels thanks to a scalpel and to empty the shell from all organic tissues.

Figure 2. Mussel valves after being dissected
Source : Alexandre Fouache (2021)

Biometric measurement

During 30 minutes, valves are weighted (BALANCE SARTORIUS, model R160P-*F1, Resolution : 0.00001). During this step, and for further process, it is important to separate the right valve and left valve to limit possible difference inherent to each side.
Length and apical asymmetry (i.e., represented by the distance between the apex of shell and its distal part) of each shell, and width and height of each valve were measured using to a digital caliper (Figure 3).
Throughout this work, weight is expressed in gram and length, width, apical asymmetry and height are expressed in millimeter.

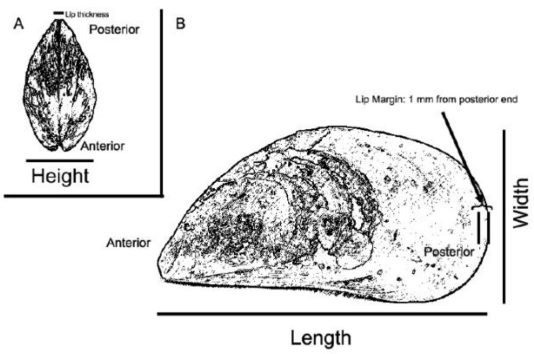


Figure 3. Biometric parameters of a mussel shell
Source : Auker (2010)
In this work, height is half the height of this figure, height of one valve and not for the complete shell because measurements were realized separately for the right and left valves.

These biometric data were further used to calculate the domedness, i.e. the ratio between the width and length of a valve.

Strength and compressibility measurement

After these step, shell crushing resistance and compressibility were measured with a dynamometer. Shell crushing resistance or shell strength represents the maximal strength that can bear a mussel valve before being broken, express in Newton. The compressibility of a valve was estimated as the ratio between the amplitude of the deformation of a valve (i.e. the difference of height of the valve without any pressure and height of the valve just before being broken) and the height of the valve.

To test shell crushing resistance and compressibility, a dynamometer (AMETEK Test and Calibration Instruments, CS225 SERIES, Chatillon Force Measurement) was used (Figure 4). To perform these tests, the dynamometer is set up to go down at a speed of 5 mm.min^-1, without any force limitation. The strength puts on shell is measure at a rate acquisition of 100Hz and data exported are composed of 400 points distributed equally along the data set of a shell. The pressure applies on shell spend 10 to 20 seconds, depending on the shell. The press stops to exercise pressure on shell when it detects an abrupt decrease in force apply on it.

Figure 4. Dynamometer AMETEK Test and Calibration Instruments, CS225 SERIES, Chatillon Force Measurement
Source : Alexandre Fouache (2021)

Statistical analysis

Morphological scaling

Before any further analyses of strength and compressibility, biometric parameter of shell and valves were studied. First, we verified that relationship between a biometric parameter and length follows an allometric relation with an equation of the type : $y = ax^b$.

Then, to conduct strength and compressibility analyses, we studied the difference between each biometric parameter of the two valves of a shell using a Wilcoxon test, to determine if there is any significant symmetry between valves. This test is realized in a confidence interval of 95%.

Strength and compressibility

About strength and compressibility, to test if there is any symmetry between valves, a modified t-test has been realized. This test is realized in a confidence interval of 95%.

Finally, to compare strength and compressibility to mass and domedness of a valve between levels on the foreshore, between study sites and between parasited and non-parasited mussels, I compared slopes and elevations of trendlines for correlated data. This test is realized in a confidence interval of 95%.

Results

Valves' symmetry

In this work, I first investigated the symmetry between valves. About biometric parameters, i.e. mass, width and length, right valve and left valve are significantly identic ($p > 0.05$) : there is a strong symmetry between valves in M. edulis.

Then, the symmetry of valves about physical parameters has been tested. The strength of the right valve is significantly greater than the strength of the left valve ($a = 0.8044$, $p < 0.05$) (Figure 5). This asymmetry is also observable for compressibility : the right valve is significantly more compressible than left valve ($a = 0.4122$, $p < 0.05$) (Figure 6).

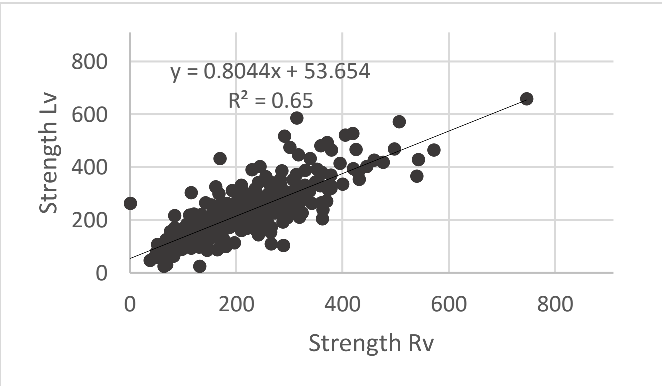


Figure 5. Strength of the left valve (Lv) compared to strength of the right valve (Rv) of mussels from Le Portel

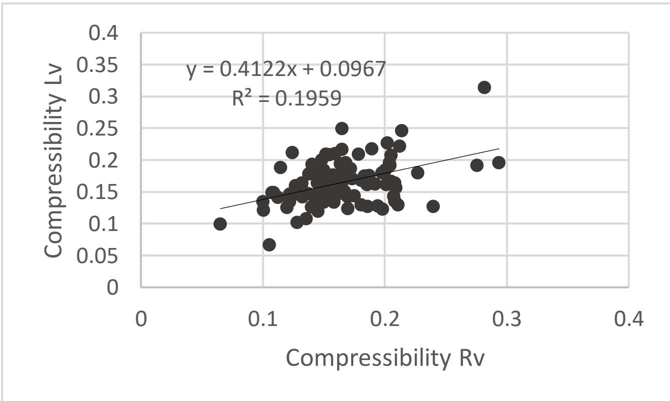


Figure 6. Compressibility of the left valve (Lv) compared to compressibility of the right valve (Rv) of mussels located on low level on the foreshore from Le Portel

These observations are the same for mussels from all levels on the foreshore and for the 2 sites.

Allometric relation in non-parasited mussels' shells

In this part, I focus on the allometric relation that exist in mussels' shell for biometric parameters. Indeed, measures reveal that there is strong allometric relations (equation of the type ax^b) between height, mass, width and apical asymmetry and length with a correlation coefficient always superior to 0.6285 (Tables 4 to 7). This significance allows, for the following parts, to compare one of these biometric parameters to the physical parameters. Here, for the sake of simplicity, I chose to keep mass.

Table 4. Allometric relation between height of a valve and its length, for each level and each site and its significance equality between levels and sites

Height	Pointe aux Oies				Equality significance	Le Portel			
Level	Equation	r²	Significance	Inference		Equation	r²	Significance	Inference
Low	$0.1629x^{1.0296}$	0.9003	**	None	≠	$0.2362x^{0.9352}$	0.8714	**	c
Medium	$0.2455x^{0.9375}$	0.8342	**	c	≠	$0.1738x^{1.0468}$	0.9005	**	c
High	$0.4147x^{0.7925}$	0.8302	**	b	≠	$0.2845x^{0.9045}$	0.9128	**	ab

* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%; a = significantly equal to low level; b = significantly equal to medium level; c = significantly equal to high level; In the equality significance column "=" and "≠" represent respectively the significant equality and the significant difference between mussels from the same level but from different site.

Table 5. Allometric relation between masse of a valve and its length, for each level and each site and its significance equality between levels and sites

Mass	Pointe aux Oies				Equality significance	Le Portel			
Level	Equation	r²	Significance	Inference		Equation	r²	Significance	Inference
Low	$0.00002x^{2.9878}$	0.9344	**	bc	=	$0.0001x^{2.5171}$	0.9119	**	None
Medium	$0.0002x^{2.3582}$	0.6285	**	ac	≠	$0.00004x^{2.9024}$	0.8721	**	c
High	$0.00009x^{2.6312}$	0.7686	**	ab	≠	$0.0001x^{2.4908}$	0.9055	**	b

* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%; a = significantly equal to low level; b = significantly equal to medium level; c = significantly equal to high level; In the equality significance column "=" and "≠" represent respectively the significant equality and the significant difference between mussels from the same level but from different site.

Table 6. Allometric relation between width of a valve and its length, for each level and each site and its significance equality between levels and sites

Width	Pointe aux Oies				Equality significance	Le Portel			
Level	Equation	r²	Significance	Inference		Equation	r²	Significance	Inference
Low	$1.0272x^{0.7761}$	0.9033	**	bc	≠	$0.8564x^{0.841}$	0.8868	**	bc
Medium	$1.3171x^{0.7087}$	0.7896	**	ac	≠	$0.8283x^{0.8539}$	0.9547	**	ac
High	$1.0246x^{0.7769}$	0.9117	**	ab	≠	$0.9917x^{0.8046}$	0.8463	**	ab

* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%; a = significantly equal to low level; b = significantly equal to medium level; c = significantly equal to high level; In the equality significance column "=" and "≠" represent respectively the significant equality and the significant difference between mussels from the same level but from different site.

Table 7. Allometric relation between apical asymmetry of a valve and its length, for each level and each site and its significance equality between levels and sites

Apical asymmetry	Pointe aux Oies				Equality significance	Le Portel			
Level	Equation	r²	Significance	Inference		Equation	r²	Significance	Inference
Low	$0.245x^{1.0362}$	0.234	**	None	≠	$0.1285x^{1.1775}$	0.3742	**	None
Medium	$0.0915x^{1.2662}$	0.6175	**	c	=	$0.0536x^{1.4601}$	0.671	**	c
High	$0.26x^{-0.7618}$	0.4554	**	b	≠	$0.2627x^{0.9621}$	0.6165	**	b

* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%; a = significantly equal to low level; b = significantly equal to medium level; c = significantly equal to high level; In the equality significance column "=" and "≠" represent respectively the significant equality and the significant difference between mussels from the same level but from different site.

To illustrate these figures, there is significantly no difference between mass compared to length of low, medium and high levels on the foreshore for mussels from La Pointe aux Oies while there is only a significant equality between medium and high level for mussels from Le Portel (Figure 7 and 8).

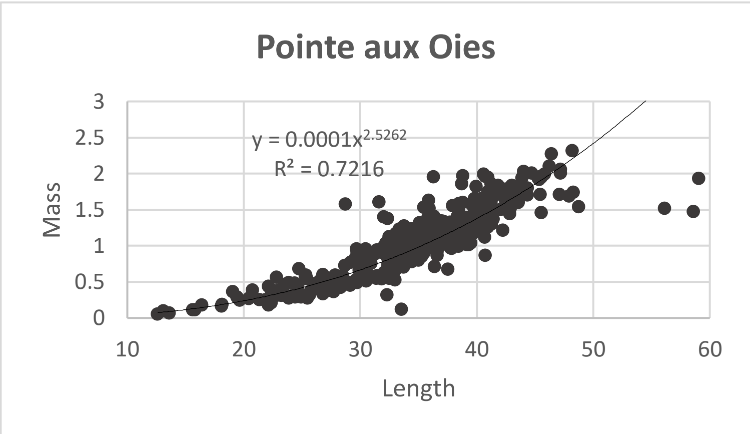


Figure 7. Mass compared to the length of mussels located on low, medium and high levels on the foreshore from La Pointe aux Oies

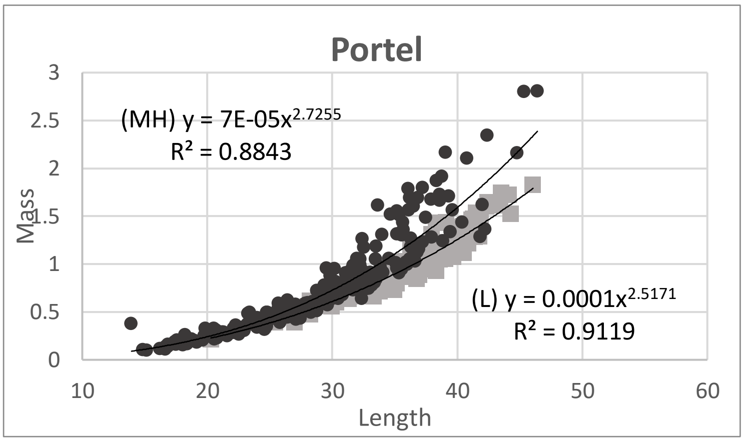


Figure 8. Mass compared to the length of mussels located on low (light grey) and medium and high (black) levels on the foreshore from Le Portel

Strength of non-parasited mussels' shells : strength compared to mass

After highlighting those biometric parameters are closely linked, further analyses can be made by studying the relation between strength and mass. We can observe globally the same behaviour of shells for mussels from La Pointe aux Oies and Le Portel : mussels from the lowest level are weaker than mussels from the medium level (Table 8).

Table 8. Comparison of strength of mussels' shell from the 2 study sites between levels on the foreshore

	Pointe aux Oies	Le Portel
Strength	Low < Medium = High	Low < High < Medium

The statistical analyses further showed that, there is a strong correlation between shell mass, and other biometric parameters, and its strength for each level, each site and each valve (Figure 9). Moreover, these data confirm, as previously highlighted, the significant difference between left and right valve strength : left valve is weaker than right valve. Eventually, there is a global significant equality of the relation between strength and mass between the 2 study sites, except for left valves of mussels from medium levels (Tables 9 and 10).

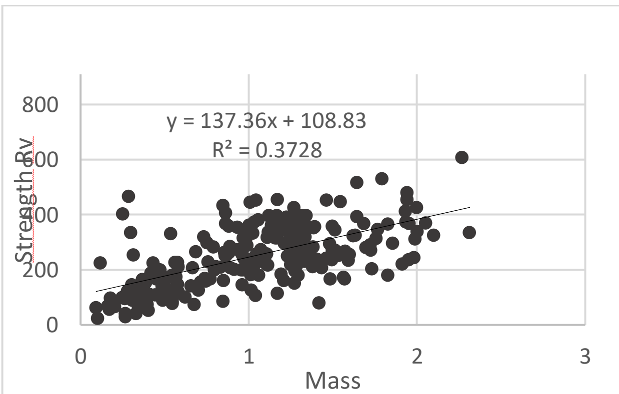


Figure 9. Strength of right valves compared to its mass located on low and high levels on the foreshore from La Pointe aux Oies

Table 9. Relation between strength of right valves and its mass, for each level and each site and its significance equality between levels and sites

Strength / Mass	Pointe aux Oies				Equality significance	Le Portel			
Level	Equation	r²	Significance	Inference		Equation	r²	Significance	Inference
Low	128.49x + 107.18	0.3345	**	c	=	146.42x + 90.277	0.4249	**	c
Medium	190.28x + 74.021	0.6565	**	c	=	235.15x + 33.405	0.7988	**	None
High	146.75x + 110.6	0.426	**	ab	=	169.69x + 68.776	0.6733	**	a

* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%; a = significantly equal to low level; b = significantly equal to medium level; c = significantly equal to high level; In the equality significance column "=" and "≠" represent respectively the significant equality and the significant difference between mussels from the same level but from different site.

Table 10. Relation between strength of left valves and its mass, for each level and each site and its significance equality between levels and sites

Strength / Mass	Pointe aux Oies				Equality significance	Le Portel			
Level	Equation	r²	Significance	Inference		Equation	r²	Significance	Inference
Low	150.17x + 85.201	0.4618	**	None	=	149.6x + 93.474	0.5494	**	None
Medium	166.96x + 112.7	0.5318	**	c	≠	244.99x + 37.23	0.8364	**	None
High	189.85x + 75.64	0.663	**	b	=	197.62x + 62.414	0.7618	**	None

* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%; a = significantly equal to low level; b = significantly equal to medium level; c = significantly equal to high level; In the equality significance column "=" and "≠" represent respectively the significant equality and the significant difference between mussels from the same level but from different site.

Strength of non-parasited mussels' shells : strength compared to domedness

To consider all biometric aspects of a shell and its role in its strength, it is interesting to study the relation between strength and domedness, i.e. ratio between width and length, of valves. The first element that highlight analyses is that there is a significant negative correlation between these 2 parameters (Figure 10). Thus, the larger a mussel is compared to its length, the weaker it is.

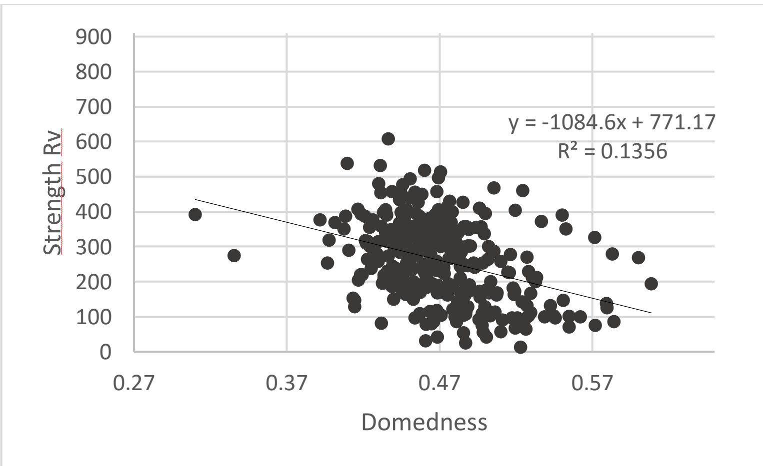


Figure 10. Strength of right valves compared to its domedness located on low, medium and high levels on the foreshore from La Pointe aux Oies

Secondly, compare to mass, domedness bring complementary information. Globally, domedness have a more important influence on strength for right valve, except for mussels located on medium and high levels from Le Portel. These exceptions seem to be one of the main reasons of the non-significant equality between mussels located on these 2 levels from the 2 sites (Table 11 and 12).

Table 11. Relation between strength of right valves and its domedness, for each level and each site and its significance equality between levels and sites

Strength / Domedness	Pointe aux Oies				Equality significance	Le Portel			
Level	Equation	r²	Significance	Inference		Equation	r²	Significance	Inference
Low	-1504.7x + 936.52	0.1483	**	bc	=	-710.47x + 582.43	0.0484	*	None
Medium	-1116.4x + 818.26	0.2224	**	ac	≠	-1893.2x + 1184.3	0.1706	**	c
High	-1169.1x + 814.52	0.1407	**	ab	≠	-424.86x + 419.29	0.0579	*	b

* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%; a = significantly equal to low level; b = significantly equal to medium level; c = significantly equal to high level; In the equality significance column "=" and "≠" represent respectively the significant equality and the significant difference between mussels from the same level but from different site.

Table 12. Relation between strength of left valves and its domedness, for each level and each site and its significance equality between levels and sites

Strength / Domedness	Pointe aux Oies				Equality significance	Le Portel			
Level	Equation	r²	Significance	Inference		Equation	r²	Significance	Inference
Low	-1272.5x + 829.73	0.1091	**	None	=	-677.76x + 572.05	0.0538	*	b
Medium	-832.35x + 693.06	0.1377	**	c	≠	-2080.2x + 1290.1	0.195	**	ac
High	-1296.6x + 884.26	0.1746	**	b	≠	-618.05x + 535.2	0.0948	**	b

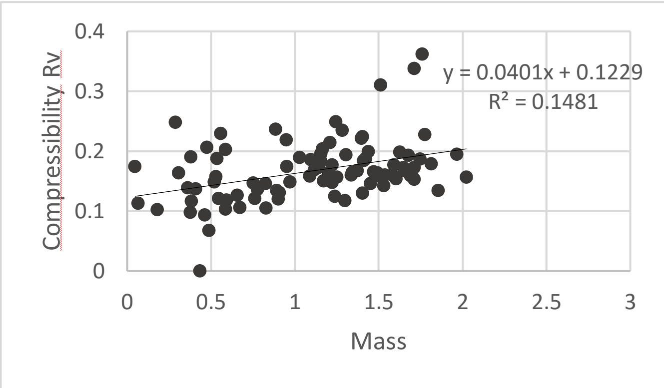
* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%; a = significantly equal to low level; b = significantly equal to medium level; c = significantly equal to high level; In the equality significance column "=" and "≠" represent respectively the significant equality and the significant difference between mussels from the same level but from different site.

Compressibility of non-parasited mussels' shells

The second physical parameter that was studied in this work is the compressibility, i.e. ratio between amplitude and height. This parameter is significantly correlated to mass only for mussels located on the medium level, for right valves from La Pointe aux Oies and for each valve from Le Portel. Moreover, this parameter is significantly correlated to domedness only for mussel located on the medium level from Le Portel (Table 13 and Figures 11 and 12).

Table 13. Global results about compressibility

	Pointe aux Oies	Le Portel
Compressibility and mass	No significant relationship for Low and High located mussels for both valves	
	Significant relationship for Medium located mussels for right valve only, equality significance between the 2 sites (Table 14)	
Compressibility and domedness	No significant relationship	Significant relationship only for Medium located mussels
		Right valve : Negative correlation Left valve : Positive correlation



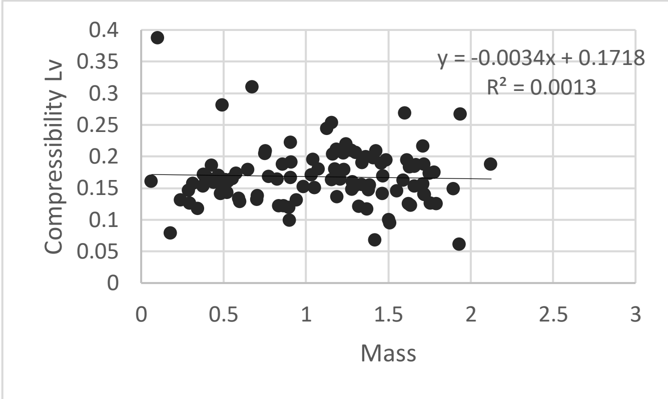


Figure 11. Compressibility of right valves compared to its mass located on medium level on the foreshore from La Pointe aux Oies : correlation coefficient is enough to have significant relation in a confidence interval of 95%

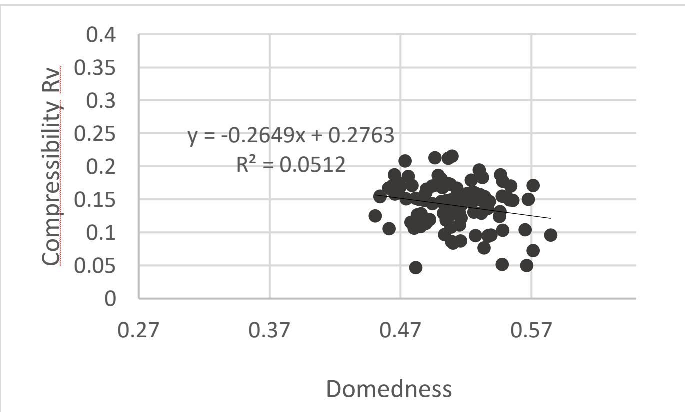
Figure 12. Compressibility of left valves compared to its mass located on medium level on the foreshore from La Pointe aux Oies : correlation coefficient is not enough to have significant relation in a confidence interval of 95%

Table 14. Relation between compressibility of right valves and its mass, for medium level and each site and its significance equality between levels and sites

Compressibility / Mass	Pointe aux Oies			Equality significance	Le Portel		
Level	Equation	r²	Significance		Equation	r²	Significance
Medium Right valve	0.0401x + 0.1229	0.1481	**	=	0.0169x + 0.1278	0.0632	**

* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%; In the equality significance column "=" and "≠" represent respectively the significant equality and the significant difference between mussels from the same level but from different site.

About the correlation between compressibility and domedness of mussels located on medium level on the foreshore, from Le Portel, there is a significant difference among left and right valves ($p < 0.05$). Indeed, compressibility of right valves and domedness are significantly correlated negatively while this correlation is significantly positive for left valves (Figure 13 and 14).



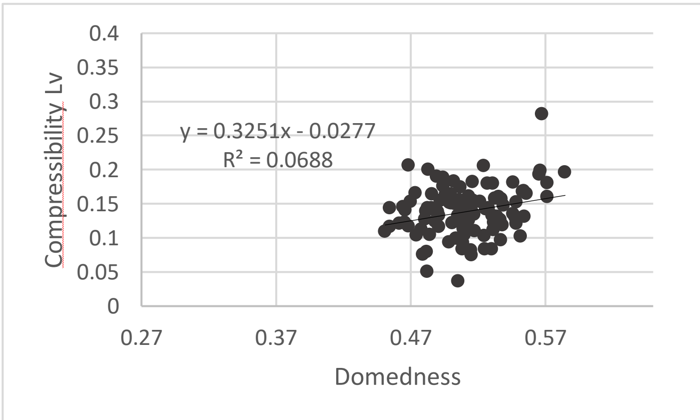


Figure 13. Compressibility of right valves compared to its domedness located on medium level on the foreshore from Le Portel

Figure 14. Compressibility of left valves compared to its domedness located on medium level on the foreshore from Le Portel

Valves' symmetry of parasited mussels

In this preliminary approach, I did not take in account the degree of parasitism, and focused on the difference between parasite and non-parasited mussels. I firstly analysed the symmetry between valves to know if it is relevant to compare parasited and non-parasited mussels. As observed in non-parasited mussels, parasited valves of a shell are significantly identic ($p > 0.05$) for biometric parameters : right and left valves of parasited mussels are symmetric, then the parasitism does not induce an asymmetry in valve biometry.

Then, the symmetry of valves about physical parameters has been tested. The strength of the right valve is significantly higher than the strength of the left valve ($a = 0.4377$, $p < 0.05$) (Figure 15). This asymmetry is not applicable for compressibility because there is no significative correlation in a confidence interval of 95%, between compressibility of each valve. Compared to asymmetry between valves of a non-parasited mussel, parasited mussels present a much larger higher asymmetry between valves as $a_{(non\text{-}parasited\ mussels)} = 2a_{(parasite\ mussels)}$.

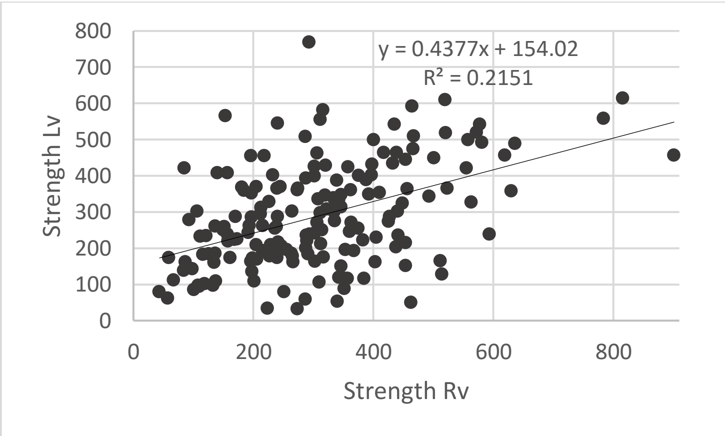


Figure 15. Strength of the left valve (Lv) compared to strength of the right valve (Rv) of parasited mussels from La Pointe aux Oies

Allometric relation in parasited mussels' shells

Here, I am interested in the allometric relation that exist in mussels' shell for biometric parameters. Analyses highlight that there is a significant allometric relations between height, mass, width and apical asymmetry and length with a correlation coefficient always superior to 0.3394 (Table 15 to 18). This significance allows, for the following parts, to compare one of these biometric parameters to the physical parameters. Here, I chose to keep mass.

Table 15. Allometric relation between height of a valve and its length, and its significance equality between parasited and non-parasited mussels

Height	Pointe aux Oies			
Level	Equation	r²	Significance	Inference
High	$0.4147x^{0.7925}$	0.8302	**	None
High parasited	$0.4603x^{0.8164}$	0.6078	**	None

* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%

Table 16. Allometric relation between mass of a valve and its length, and its significance equality between parasited and non-parasited mussels

Mass	Pointe aux Oies			
Level	Equation	r^2	Significance	Inference
High	$0.00009x^{2.6312}$	0.7686	**	None
High parasited	$0.0005x^{2.2889}$	0.6852	**	None

* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%

Table 17. Allometric relation between width of a valve and its length, and its significance equality between parasited and non-parasited mussels

Width	Pointe aux Oies			
Level	Equation	r^2	Significance	Inference
High	$1.0246x^{0.7769}$	0.9117	**	None
High parasited	$1.2116x^{0.7448}$	0.7328	**	None

* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%

Table 18. Allometric relation between apical asymmetry of a valve and its length, and its significance equality between parasited and non-parasited mussels

Apical asymmetry	Pointe aux Oies			
Level	Equation	r^2	Significance	Inference
High	$0.26x^{-0.7618}$	0.4554	**	None
High parasited	$0.0915x^{1.3493}$	0.3394	**	None

* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%

These figures highlight those biometric parameters are globally higher for parasited mussels than for non-parasited mussels compare to length. This is particularly remarkable about mass (Figure 16).

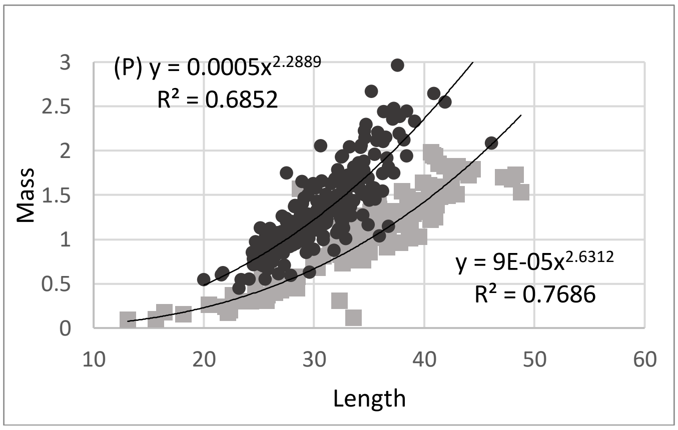


Figure 16. Mass compared to length of parasited mussels (P, black) and non-parasited mussels (light grey) from La Pointe aux Oies

Strength of parasited mussels' shells

After highlighting biometric parameters are linked, further analyses can be made about the relation between strength and mass. The main result that these analyses provide is that parasited mussels are significantly weaker than non-parasited mussels ($p < 0.05$) (Figure 17).

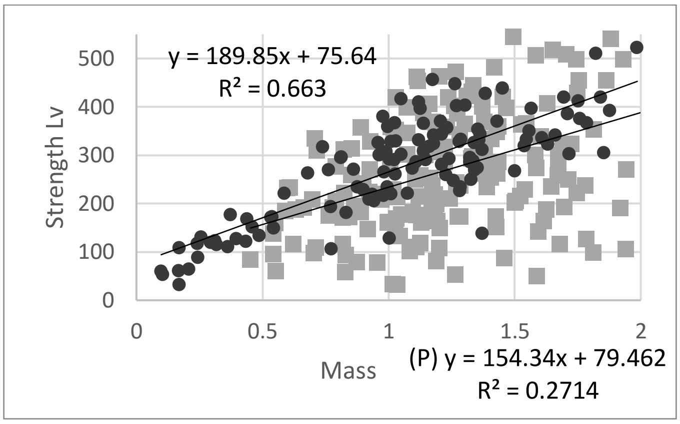


Figure 17. Strength of left valve compared to its mass of parasited mussels (P, light grey) and non-parasited mussels (black) from La Pointe aux Oies

Analyses highlight that there is a strong correlation between mass of the shell and its strength for parasited mussels (Table 19 and 20).

Table 19. Relation between strength of right valves and its mass and its significance equality between parasited and non-parasited mussels

Strength / Mass	Pointe aux Oies			
Level	Equation	r²	Significance	Inference
High	146.75x + 110.6	0.426	**	None
High parasite	189.43x + 47.125	0.3721	**	None

* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%

Table 20. Relation between strength of left valves and its mass and its significance equality between parasited and non-parasited mussels

Strength / Mass	Pointe aux Oies			
Level	Equation	r²	Significance	Inference
High	189.85x + 75.64	0.663	**	None
High parasite	154.34x + 79.462	0.2714	**	None

* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%

Moreover, there is no significance between strength and domedness for parasited mussels. So, an effect of this parasite is to lead to an independence between these two parameters.

Compressibility of parasited mussels' shells

In this work, I studied the relation between strength and compressibility and biometric parameters. In this part, I am interested in compressibility. In contrast to what has been observed in non-parasied mussels, parasitism leads to an independence between compressibility and mass and compressibility and domedness for right and left valves. Indeed, there is globally no significant correlation in a confident interval of 95% between compressibility and biometric parameters for non-parasited mussels. However, for parasited mussels, there is no significant correlation in a confident interval of 95% between compressibility and biometric parameters at all.

Discussion

Valves' symmetry

In a previous work conducted on the shell resistance of the California mussels, *Mytilus californianus* (Crane & Denny, 2020), there was a relation between strength difference between right and left valve and width difference between right and left valve. However, this relation is not applicable to Mytilus edulis. Indeed, I showed that right and left valves are biometrically symmetric but asymmetric when it comes to their strength. Environmental conditions of development can be the main cause of this difference by leaving available elements, that are needed for mussels development, in different proportions, or by presenting different physical conditions that influence directly mussels development. Moreover, this difference can be explained by another reason, that could explain difference between right and left valves strength and compressibility (Tables 21 and 22). I think that mussels from genus *Mytilus* can have an asymmetric embryologic development. *For M. edulis*, my hypothesis is that right valve could develop first and this would explain that left valve is weaker and less compressible.

Table 21. Relation between strength of right valves and strength of left valves for each level and each site and its significance equality between levels and sites

Strength between valves	Pointe aux Oies				Equality significance	Le Portel			
Level	Equation	r²	Significance	Inference		Equation	r²	Significance	Inference
Low	0.6998x + 72.636	0.4786	**	bc	=	0.5575x + 111.74	0.4293	**	bc
Medium	0.6977x + 101.03	0.469	**	ac	=	0.8918x + 33.116	0.7701	**	ac
High	0.6675x + 101.38	0.4342	**	ab	=	0.7841x + 59.873	0.5575	**	ab
High parasited	0.4377x + 154.02	0.2151	**	None					

* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%; a = significantly equal to low level; b = significantly equal to medium level; c = significantly equal to high level; In the equality significance column "=" and "≠" represent respectively the significant equality and the significant difference between mussels from the same level but from different site.

Table 22. Relation between compressibility of right valves and compressibility of left valves for low and high levels and each site and its significance equality between levels and sites

Compressibility between valves	Pointe aux Oies				Equality significance	Le Portel			
Level	Equation	r²	Significance	Inference		Equation	r²	Significance	Inference
Low	0.2834x + 0.1066	0.0953	**	b	≠	0.4122x + 0.0967	0.1959	**	None
High	0.3148x + 0.0976	0.1015	**	a					

* : significance in a confidence interval of 95%; ** : significance in a confidence interval of 99%; a = significantly equal to low level; b = significantly equal to medium level; c = significantly equal to high level; In the equality significance column "=" and "≠" represent respectively the significant equality and the significant difference between mussels from the same level but from different site.

This asymmetry between right and left valves about strength is also relevant about compressibility. Indeed, right valve domedness and compressibility are negatively correlated while left valve domedness and compressibility are positively correlated. If there is an asymmetry in the development during embryologic stage, it could explain this fact too. Eventually, an analyse of the composition of each valve could be interesting to understand why there are these differences.

However, under the hypothesis that the observed asymmetry is due to embryologic development, this work indicates that parasitism increases this difference. But parasited mussels are essentially the oldest one. So mussels are not parasited at embryologic stage but parasitism increase asymmetry between valves that would happen during embryologic development.

Correlation between parameters

This work provides many information about correlation between biometric and physical parameters (Figure 18). This work, stresses that compressibility globally does not depend on biometric parameters. Indeed, La Pointe aux Oies and Le Portel are two sites with different environmental parameters. Moreover, there are differences of correlation between compressibility and biometric parameters between sites. Thus, compressibility must depend on other parameters that would be essentially environmental parameters.

	Pointe aux Oies Low	Pointe aux Oies Medium	Pointe aux Oies High	Pointe aux Oies High parasited	Le Portel Low	Le Portel Medium	Le Portel High
Apical asymmetry							
Half-height / Length							
Mass / Length							
Width / Length							
Compressibility Rv / Compressibility Lv							
Strength Rv / Strength Lv							
Compressibility Lv / Domedness							
Compressibility Lv / Mass							
Compressibility Rv / Domedness							
Compressibility Rv / Mass							
Strength Lv / Domedness							
Strength Lv / Mass							
Strength Rv / Domedness							
Strength Rv / Mass							

Figure 18. Correlation between each parameters for mussels from each level and each site
Significant positive and negative correlations are shown in light and dark grey, respectively. Non-significant correlations are shown in black.

Moreover, compressibility must depend on other parameters than biometric parameters. However, compressibility and strength are globally correlated (Figure 19). This observation suggests that the strength of a valve is fundamentally influenced by environmental parameters.

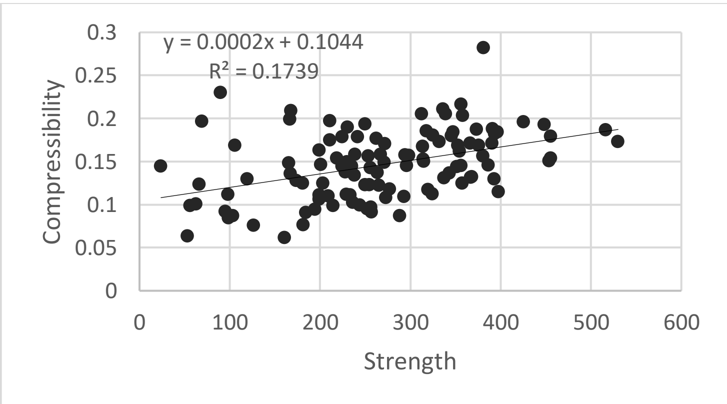


Figure 19. Compressibility of right valves compared to its strength located on high level on the foreshore from La Pointe aux Oies

Impact of weaker shells on low level

This work highlights that low level located mussels are weaker than other mussels. Mussels could need an immersion period but an emersion period too to develop well that could explain that medium level located mussels are more strength than others. This fact induces a competition between men and predators. Indeed, in one hand, mussels fishermen harvest mussels from low level, because low located mussels are bigger, cleaner and less parasited. On the other hand, predators adapt their behaviour to save energy. Thus, crabs and bird hunt mussels were breaking their shell is the easiest : in low levels. This is confirmed by the fact that there are consistently many birds at low level on the foreshore of La Pointe aux Oies (Laurent Seuront, personal communication, June 2021). Face to that, low level located mussels will be harvested more than those of the other levels and their number will progressively decrease. Moreover, in the world, there is climate change and calcium carbonate is more soluble in cold water. This increase in precipitation of calcium carbonate facilitates the assimilation of calcium by organisms as mussels that will increase progressively their shell strength and by the same way, their compressibility (Vermeij, 1980). These changes will probably modify this dynamic with time by increasing strength of low level located mussels and this will, by the same time, change predators' behaviour.

Parasited mussels and degree of parastism

Parasitism causes an increase of porosity in mussels' shell. However, parasited mussels are heavier than non-parasited one. This mean that parasited mussels allocate more energy to develop and to repair their shell than non-parasited one. Moreover, parasitism appears to lead to an independence between domedness and physical parameters. This leads to separate parasited mussels by their parasitism degree.
First of all, parasited mussels can be classed in 5 classes : A to E (Figure 20). A degree regroup non parasited mussels : here, I am not interested by this class. Then, B degree represent parasited mussels which have their periostracum removed at less than 50%. C degree represent parasited mussels which have their periostracum removed at more than 50%. Then, D degree regroup mussels that present furrows dig by this parasite. Finally, E degree regroup mussels that are so weak that strength to maintain shell closed is enough to break it, main at level where adductor muscle is implanted. La Pointe aux Oies shelter mainly B and C classes of parasited mussels.

Figure 20. Classification of parasited mussels
Source : Seuront, personal communication (2021)

Difference between B and C classes of parasited mussels have been assessed. This leads to the conclusion that there is no difference of correlation of parameters between global results, results about B class and about C class. There is significatively no difference ($p < 0.05$) between B-classed and C-classed parasited mussels (Figures 21 and 22).

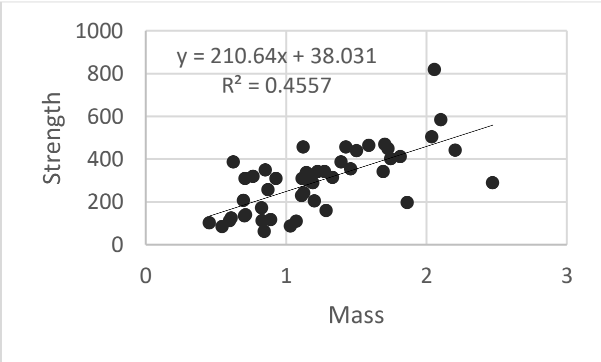


Figure 21. Strength of right B-classed valves compared to its mass located on high level on the foreshore from La Pointe aux Oies

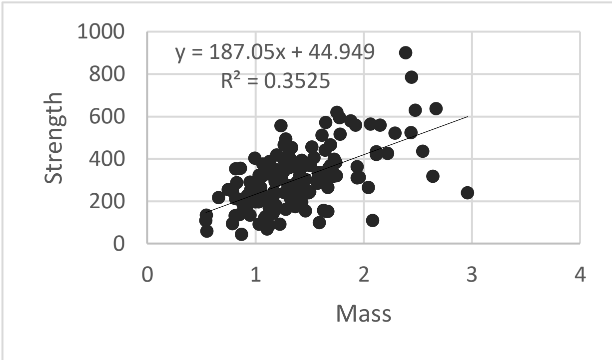


Figure 22. Strength of right C-classed valves compared to its mass located on high level on the foreshore from La Pointe aux Oies

Conclusion

As a conclusion of this work, I showed that even if low level located mussels can eat and calcify more than other mussels, they are not the strongest but are actually the weakest. The site of Le Portel present stronger mussels on medium level. These observations could not have been verified about compressibility because of data sets that were not significantly correlated. However, parasited mussels are present only the site of La Pointe aux Oies and located on the higher levels. So the third hypothesis is verified : the most a mussel is submerged, the least its shell is parasited, even if it is not due to an higher resistance. Finally, this work showed that, compared to mass, a parasited mussel is weaker than a non-parasited mussel, independently of its degree of parasitism.

Moreover, this work confirms that in all environments studied, there is a strong correlation and a significant allometric relation between biometric parameters that are height, length, mass and width and a symmetry of right and left valves of a shell. Although this symmetry about biometric parameters are proved in this work, it highlight another important fact : left valves are significantly weaker and less compressible than right valves. It would be interesting in the future to analyse the structure of each valve to determine if there is a difference in its conception that can explain this weakness of left valves compare to right valves.

Then, about parasited mussels, this work highlights that, even if parasitism leads to an increase in shell porosity, for the same length, parasited mussels are heavier than non-parasited mussels. This can be explained by an increase of energy allocated to shell repair. Finally, parasitism leads to an independence between strength and domedness of shell.

In the future, to complete this work, it can be interesting to compare these data to those of mussels from Berck-sur-Mer. Indeed, its domedness is much higher than mussels from La Pointe aux Oies and Le Portel. Moreover, a test-experiment on mussels from Berck suggest that, compare to mass, shells from Berck-sur-Mer are weaker than others.

Reflexive feedback

This internship was very formative. Indeed, the different parts that compose this work are relevant of the process that I follow during these 12 weeks. Polytech promote greatly the scientific works and reflexion. However, I discover during this internship that this scientific reflexion has more dimension than what we learn and that there is several important reflexes to apply at each step : mainly to separate the work in different parts and to progress gradually by starting by general issues to reduce the particular issues that the problematic ask.

I apply several skills that I get in Polytech'Tours. First, during these 2 years, I read and resume many scientific articles. This allowed me to read a consequent number of articles during the beginning of the internship familiarize with this subject that is not a main point of my formation. Then, I realized statistical tests on results that I got. However, these statistical tests were my main problem during the internship. Indeed, I had not enough basis in that field to apply efficiently this method. So this step took me 2 weeks and a half and I realized 3 different tests to achieve to get coherent results.

Moreover, as the experimental work was very repetitive, to avoid error due to a loss of focus, I elaborate some reflex that allow a decrease in focus during experiment without make any error. For example, all shells were aligned in a precise order to complete exactly excel sheet or I always started to treat right valve of a mussel and left valve after.

This internship also allowed me to test in real condition my English level. Indeed, at the end of it, I presented my results to 3 researchers that work one the project SOLACE. In these 3 researchers, 2 are English-speakers so my presentation was in English.

Where many of my trainee colleague had many equipment problem, I had the chance to only use two electronic equipment : a dynamometer and a digital caliper. That gives hope that I had not encounter any of these problems. Unfortunately, I had many problems on digital caliper (battery problem, display problem, or humidity problem). This leads sometimes to a non-respect of 5 to 10 minutes in the time manipulation of mussels for the measurement step, until I solve the problem. This is a first margin of error in results presented in this work.

A second margin of error is the fact that, sometimes, dynamometer detected a brutal decrease in strength that it applies. The consequence of that is mussel shell was not broken and I could not retry this measurement on this valve : literature proved that it exists a shell fatigue. So I eliminated manually these data.

If I had one think to change in this internship is time I spend on data treatment. Indeed, I wanted to achieve too fast the different part of this step. This leads to some errors, not serious and irreversible problems, but enough to have to restart one or two parts, that leads to a certain waste of time, that did not exceed 2 or 3 days. However, these errors were the consequence of my lack of experience in a research context, which I considerably improved throw the work described in this work.

About my projection in a job, before doing this internship, I was attracted by science and research. This internship confirms this wish to continue in field of research. I already inquired about a continuation in a thesis after the obtention of my engineer diploma to Ms Boisneau and Mr Rodrigues that I greatly thanks. Today, I do not know exactly in what field I will precisely looking for a thesis : I know that it would be in aquatic ecosystem but I am open-minded, animals, vegetal or sedimentologic parts interest me, even if I think I have a preference for animals field. Eventually, in this project, I know that I will, in the next day, start to work on the R language that is important to master in research field.

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Titre : compressibilité et résistance à l’écrasement de la coquille de la moule commune *Mytilus edulis* : effets du temps d’immersion et du degré de parasitisme

Résumé : In this work, I attempt to precise the effect of the immersion time and of the parasitism on mussels shell strength and compressibility. To achieve it, I studied mussels *Mytilus edulis* from two different sites : La Pointe aux Oies and Le Portel. After established strong allometric relation between biometric parameters that prove that valves are symmetric, I studied the relation between these biometric parameters and strength and compressibility of valves. This work highlight left valves are weaker and less compressible than right valves and strength of a valve, compare to compressibility, is highly correlated to biometric parameters. Indeed, compressibility is correlated to mass or to domedness (ratio between width and length) only for mussels localised on medium levels of the foreshore. Then, I proved that low level located mussels have weaker shell than those of high or medium levels. Moreover, parasitism leads to an increase of porosity of mussel shell. However, this work highlights that, at a same length, parasited mussels are heavier than non-parasited mussels. Eventually, I highlight that parasited mussels, with allometric relation comparable to that of non parasited-mussels, are weaker than non-parasited mussels.

Mots Clés : Mussel, shell, strength, compressibility, allometry, parasitism, valve

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Annex I : State-of-the-art work

Problem	Species	Answer
• Mechanical resistance of shells links to differences in shell build and microstructure	*Nucella lapillus*	• Tropical individuals are strengther than temperate due to less solubility of calcium carbonate • Some changes in microstructure • Tropical : spherical and better developed cross-lamellar structures
• Does the refuge-seeking behaviour of blue mussels and transmission interference by oysters affect parasite infection levels in blue mussels ? • Does the resulting effect on parasite infection levels differ between parasites with direct and complex life cycles ? • Do the position in the matrix and parasite infection intensities affect mussel condition ?	*Crassostrea gigas, Mytilus edulis, Mytilicola spp.* (copepod*), Renicola roscovita* (Trematode)	• Refuge seeking behaviour in the oyster matrix : Reduction of infections with parasitic copepods but increase exposure to trematodes. • Result of complex abiotic and biotic mechanisms related to the position of mussels in the oyster matrix apply to the scale of oyssel reefs. • Direct effects on parasites : Transmission interference or parasite spillover
• Relationship between morphological defences and behavioural response to the threat of predation • Effect of vulnerability to the strength of intraspecific behavioural responses	*Lymnaea stagnalis, Physa fontinalis, Anisus vortex, Planorbis planorbis, Lymnaea peregra, Pimephales promelas, Culea inconstans*	• Trait compensation : morphological defences and behavioural response are negatively correlated • Thiner shells' individuals present more behavioural response
• Evaluation in shell crushing resistance among various morphs	*Apodemus sp., Turdus merula, Turdus philomelos, Cepaea nemoralis*	• Differences of resistance between different colors • Shell strength differed between shell areas usually attacked by birds and mice : adaptation to predators
• Patterns of prey selection on gastropods • Size related predator and prey traits that influence encounter rates, attack probabilities and capture successes	*Viviparus georgianus, Promenetus exacuous, Helisoma campanulata, Helisoma anceps, Gyraulus deflectus, Gyraulus parvus, Valvata tricarinata, Amnicola limosa, Amnicola walkeri, Marstonia lustrica, Lepomis gibbosus*	• Encounter rate increase with size prey • Attack probability is better for a medium value of size • Capture successful decreases with size
• Do the four species have similar resistance to crushing ? • Is resistance to crushing a simple function of shell's morphology and morphometry ? • Are measured resistance levels comparable to the impact forces	*Patella intermedia, Patella rustica, Patella ulyssiponensis, Patella vulgata*	• Resistance to crushing is not a simple function of shell morphology and morphometry, and appears to be mainly determined by shell deformability • The resistance to crushing largely exceeds the values reported in the literature for pressures exerted by ocean waves

commonly observed in nature on rocky shores ? • Is there a direct relationship between the habitat and distribution of each species in the intertidal zone, and the observed levels of resistance to crushing ?		• No direct relationship between habit preferences-resistance to crushing
• Examination of the combined effects of warming on the morphology, shell composition and surface area • Examination of the resulting effects on mechanical strength and flexibility.	*Haliotis iris, Mytilus edulis, Arctica islandica*	• Warming by 4°C had a detrimental effect on shell strength which was associated with a reduction in shell surface area • Indirect effect on shell strength via the re-allocation of energy resources away from costly biomineralization processes in order to support higher maintenance costs • Seawater acidification : no effect on shell strength but reduce shell flexibility
• Relation between inducible defences and predation intensity	*Carcinus maenus, Mytilus edulis*	• Phenotypic plasticity • Presence of predators or of broken individuals : increase of shell resistance • Inducible defences are geographically and taxonomically widespread
• Examination of spatial variation in crushing resistance and shell pigmentation • Test effects of abiotic or biotic variables account for spatial patterns of crushing resistance and shell pigmentation • Examination of variation in primary productivity accounts for small-scale variation in these defensive traits	*Herichthys minckleyi, Mexipyrgus churinceanus*	• Variation at small spatial scales occur, often associated with habitat differences in primary productivity and substrate coloration • These local geographic differences may result from among-habitat variation in how resource productivity interacts to promote escalation in prey defences
• Comparison between the length-weight allometric relationships, shell crush resistance, attachment strength and energy storage along the body length increase • Studying the dynamics of development of both species, which can contribute to explaining the ongoing displacement of the zebra by quagga mussel	*Dreissena polymorpha, Dreissena rostriformis bugensis*	• Zebra mussels : small, high attachment strength, high shell crush resistance • Quagga mussels : large, less attachment strength and shell crush resistance than zebra mussels • Quagga mussels survives better than zebra mussels because it has better energy storage
• How shell thickness changed during the first 20 years of zebra mussels' invasion of the Hudson River ?	*Dreissena polymorpha*	• Shells in the Hudson River getting heavier and thicker over time
• Test the relation between aspect ratio and shell crush resistant • Estimate the influence of temperature on the calcification process along geographical gradients • Understand the relation between differences in exposition to swell and degree of calcification	*Autrolittorina spp., Nodilittorina spp., Littorina spp., Afrolittorina spp., Echinolittorina spp.*	• Shell with large aspect-ratio have better crush resistant than shell with small aspect-ratio • Manipulation by predators is easier with a larger shell aspect-ratio and with a smooth morphology • Higher temperature is, higher is the precipitation of calcite and easier the assimilation of this calcite is by organism • Less calcification with high exposition to swell

• Evaluate effect of the polymorphism of colour and texture on calcification		• Smooth phenotype : more resistant to crush than non-smooth phenotypes
• Define an architectural continuum of shell form • Test for an adaptive trade-off between forms	*Carrasius auratus, Lepomis gibbosus, Cyprinus carpio, Orconectes obscurus, Physa fontinalis*	• Rotund shells resisted greater crushing force, whereas elongate shells were less penetrable
• Does endolithic species richness differ among bioregions ? • Does the identity of the host drive parasite diversity ? • Do the rates of cyanobacteria infestation and mortality due to infestation differ across bioregions ?	*Mytilus galloprovincialis, Perna perna*	• Endolithic species identity differed among bioregions, but with little effect on overall species richness • The host identity was not linked to parasite diversity • The mortality due to cyanobacterial infestation and endolith-induced mortality differed among bioregions
• Test the correlation between concentration of calcite, shell lengthening, crush resistance and mortality	*Dreissena polymorpha*	• Positive correlation between concentration of calcite, shell lengthening and crush resistance : the effect is more visible with a low concentration of calcite • Positive correlation between crush resistance and mortality
• Does fatigue occur on ecologically relevant time scales ? • What morphological features are associated with strength and fatigue resistance ? • How do different loading regimes contribute to fatigue ?	*Mytilus californianus*	• On short time scales, fatigue allow some predators to fracture otherwise inaccessible prey • On long time scales, moderate episodic forces fatigue shells, which could prove lethal, depending on the mussel's capacity for repair • Valves that were larger and more domed tended both to be stronger and to withstand a longer duration at a given force
• Examination of the relationship between shell morphology, shell strength, and behavioural avoidance	*Carcinus maenas, Gibbula cineraria, Gibbula umbilicalis, Littorina spp., Osilinus lineata*	• Flat, disc-like shells took longer for crabs to handle and consume than did those with shells with taller spires • Pointed shells showed greater responsiveness to predation cues : trait compensation
• Do juvenile blue crabs exhibit a distinct preference for either micrograzer snail species in laboratory feeding trials ? • How does selectivity vary with the relative availability of prey sizes between seasons? • Can observed prey selectivity be explained through considerations of feeding efficiency ? • Are laboratory feeding preferences corroborated by field diets ?	*Bittiolum varium, Astyris lunata, Callinectes sapidus*	• *Bittiolum varium* was more profitable than *Astyris lunata* • Profitability increased for snails larger than 2 mm in length • The strength of this preference varied with crab size and season : medium sized crabs selected *Bittiolum varium* to a greater degree than did small and large crabs • The seasonal effect likely resulted from an interaction between snail species and snail size in relation to feeding efficiency • Laboratory feeding preferences corroborated by field diets
• How does the strength of a valve change with compression rate ?	*Mytilus californianus*	• A valve's time-dependent strength can influence the foraging strategies of predators trying to fracture those valves

• How does compression rate affect the susceptibility of the valve to fracture by predators ?		• Compression rate and fatigue are negatively correlated • Shell resist better to tension than compression • Shell breaks with a higher compression rate when its apply slowly : positively correlated to shell length
• Studying the spatial variation in predatory structures as well as prey defensive traits • Studying the trait matching in some areas and trait mismatching in others mediated by gene flow	*Herichthys minckleyi, Nymphophilus minckleyi, Mexithauma quadripaludium, Mexipyrgus churinceanus*	• Lack of spatial autocorrelation and phylogenetic signal in shell crushing resistance and molariform relative frequency • Reduction of frequency of molariform fish in populations in which shells are hard to crush • Local biotic conditions may play an important role in shaping predator-prey arms race
• Studying the expression of behavioural and morphological defence adaptations • How these adaptations co-vary along a gradient of intraspecific competition ?	*Radix balthica, Tinca tinca*	• Plastic defensive traits affect fitness values over a resource gradient • Modification of energetic allocation • Reduction of growth when presence of predators : less choiced by predators (feeding efficiency), increase in shell thickness
• Studying the cause of predator-induced shell thickening • Test on induced thickening	*Nucella lamellosa, Radix balthica*	• Shell thickening is homogeneous and heterogeneous • Predator-induced shell thickening is a passive consequence of reduced feeding • Thickening leads to an increase in structural strength
• Studying the sub-lethal shell damage and subsequent shell repair • Studying the role of its main intertidal predator in fragmentation of the shells	*Larus dominicanus, Himanthalia elongata, Nacella spp.*	• Repaired shell fractures are caused by rolling stones and blocks of ice in the intertidal or by tumbling in the intertidal of dislodged animals
• Does the presence of a scar in the shell of a gastropod weakens the shell relative to that of an unscarred individual ?	*Littorina spp., Conus sponsalis*	• Number of scar on shell does not affect its crushing resistance • Fragility mainly along sutures and growth lines
• Studying the combined and isolated effects of trematode infection and high temperatures during low tide air-exposure	*Mytilus edulis*	• Infected mussels survive better in warm temperatures (35°C) but worse in colder water (15°C)
• Test the relation between shell infections, effect on the shell strength and the feeding behaviour of the crab *C. maenas*	*Polydora ciliata*	• Reduction of shell strength • *Carcinus maenas* preferentially consumed periwinkles infected with *P. ciliata*
• Studying the effects of a range of water temperatures on larval parasitism of thick-shelled river mussel	*Unio crassus*	• Water temperature during the parasitic phase plays an important role in development and metamorphosis of the parasite • Optimum temperature for breeding of *Unio crassus* on *P. phoxinus* : 17 °C, highest metamorphosis success and the lowest host mortality

Annex II : Species studied in the state-of-the-art