Effect of molt on the homarid lobster's life cycle

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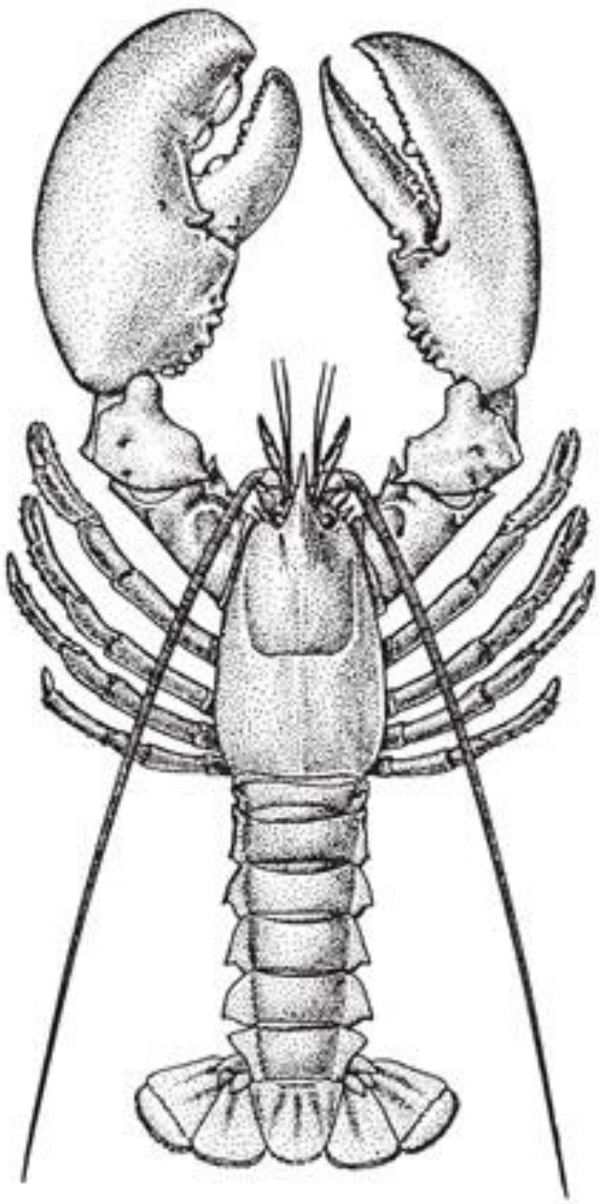

Summary

The molt cycle influences a lobster's life cycle in many ways, because a lobster is either preparing for ecdysis or recovering from it. There has been done a lot of research regarding this subject on the Homarid lobster, in order to understand and improve conservation. The molt cycle is divided in 5 major stages and several sub stages, which are used in this thesis and are adapted for *Homarus americanus.* The Homarid lobster undergoes a metamorphosis in the molt from the third to the fourth stage, it is now possible for the homarid lobster to settle on the ocean floor. For a better understanding of the mechanisms for molt, hormonal control has been intensively studied. The opinions differ and change due to new research. The mechanisms that cause molt and the internal changes ensure alterations in the homarid lobster's behavior. Observations have shown that alteration in aggression and the escape response occur over the molt cycle. Behavioral changes are seen in the mating act as well during the molt cycle. Females are affected by molt, because receptivity peaks after ecdysis and fertilization is most likely to occur during this time. More recent studies have shown that females or not that reliant on their stage in the molt cycle as was once thought. Sperm can be stored and batches of eggs can be fertilized in multiple years. Male homarid lobster's are affect by molt during the mating season through their dominance status. A dominant male loses his status when he molt, but this is regained several days afterwards. It is therefore not smart for a male to molt during the mating season, because females prefer dominant males.

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Introduction

A homarid lobster's rigid exoskeleton, consisting of partly dead material, cannot increase in size through growth, periodic molt is therefore necessary (Waddy *et al*., 1995) . During two consequent molts a homarid lobster spends 70% of the time on recovering from or preparing for the next ecdysis. During its life the American lobster is thus almost continuously physiologically preparing for the next molt or recovering from the last molt. The whole molt cycle therefore influences the American lobster's life cycle and in effect potentially alters metabolism, endocrinology, behavior and reproduction either directly or indirectly (Cromaraty *et al.*, 1991; Phillips *et al., 1980*). In this thesis these influences are studied and summarized in an attempt to understand how a homarid lobster's life cycle is affected by molt.

There has been done a lot of extensive research on homarid lobsters, not only for their commercial aims, such as fisheries, also their physiology, biochemistry and neurobiology have gained a lot of attention (Cobb & Philips, 1980). The biomechanics of lobster locomotion has also been studied, aiming at the production of underwater mine-seeking robots based on the lobster walking model (Ayers *et al.,* 1998). Interest in intensive lobster culture peaked during the 1970s (Waddy *et al*., 1995). During this time attempts were made to culture lobsters under intensive conditions, because they were very valuable on the international market (Aiken & Waddy, 1995). This indicates that lobsters are regarded of great value to humans, for purpose of consumption as well as for understanding neurobiological and muscle mechanics

This thesis will be divided in two sections namely the mechanisms and behavior. In the mechanisms section, an overall overview of how the molt cycle works and what the different stages are will be given. Here also the endocrine control of the molt cycle will be covered including a more in depth section about the metamorphosis and concluding with several molt affecting factors. The second section covers the behavior and especially the changes in behavior during the molt cycle which are related to it, including aggression and escape behavior and the influence of molt on mating.

With the gathered information from both sections the observed behavior during the molt cycle and how this proceeds from the internal changes will be discussed. Therefore my main question of this thesis is: What are the internal changes during the molt cycle and how do they alter the homarid lobster's behavior?

Molt mechanisms

In this section the mechanisms of the molt cycle will be covered in an overview of the different stages in the molt cycle, the hormonal changes involved and several factors which can influence the molt cycle. This section is meant as a prerequisite to understand behavioral changes, which will be discussed in the second part of this thesis.

Different stages

A repetitive event in a homarid lobster's life is molting, allowing the animal to grow. Drach (1944) divided the molt cycle in four basic periods, namely post-molt, inter-molt, pre-molt and molt. He also described five major stages, A-E and several sub stages, this system gained acceptance and was redefined and modified somewhat by Drach (1944) and Drach and Tchernigovtzeff (1967) (Aiken, 1980). The four basic periods are subdivided as follows: post-molt or post-ecdysis encompasses stages A, B, and C_1 through C_3, inter-molt and its synonym met-ecdysis refers to stage C_4, stage D is known as pre-molt or pr-oecdysis and molt or ecdysis revers to stage E (Waddy *et al*., 1995). This system was adapted for use with lobsters (Aiken, 1980). Table 1 shows the characteristics of the standard molt stages for *Homarus americanus*.

Stage	General characteristics	Duration (%)	Pleopod changes
A_1	Body flaccid; continuing water absorption; new dimensions achieved 4-8 hr. after ecdysis	0.3	Rows of large cells visible
A_2	Integument soft; mouthparts and tips of chelae hard; able to eat exuvia; mineralization of exocuticle begins	1.1	Rows of large cells visible
B	Integument flexible; secretion of new endocuticle commences; gastrolith exocuticle completed	2.0	Rows of large cells visible
C_1	Integument flexible; chelate appendages hard; active foraging begins; thin-lamina endocuticle forms in the merus of the cheliped; gastrolith endocuticle secretions begins	6.0	Scattered large cells visible
C_2	Carapace rigid posteriodorsal to rostrum but flexible elsewhere; thin-lamina endocuticle forming in anterodorsal carapace	7.6	Very few large cells present
C_3	Branchial carapace easily depressed by finger pressure, but rigid elsewhere; thin-lamina endocuticle forming in all areas of carapace	9.0	Normal appearance
C_4	Membranous layer formed; all parts of carapace rigid; organic reserves accumulate	30	Normal appearance
D_0	Passive premolt; may remain in this stage for extended period (anecydysis); epidermis retracts from cuticle; limb regenerates form at site autonomy; gastrolith calcification commences	≥26.0	Epidermis retreats from tip; pleopod stages 1.0-2.5
D_1	Active premolt (irreversible); new epicuticle forms and new setae invaginate to maximum depth	9.0	Setae invaginate; pleopod stages 3.0-4.0
D_2	New exocuticle formed	7.0	Setal shafts completed; pleopod stages 4.0-5.0
D_3	Extensive reabsorption of minerals from exoskeleton; ecdysial sutures and dorsal surface of merus decalcified; gastrolith exocuticle forms	2.0	Cuticular ripples form; pleopod stage 5.5
E	*Passive phase* of ecdysis (may be delayed); increased water absorption; ecdysial sutures open but thoraco-abdominal membrane is intact *Active phase* (irreversible); thoraco-abdominal membrane ruptures; carapace is thrown forward; about 15-20 min required for emergence		

Table 1. Molt stages of *Homarus americanus* (Aiken, 1980).

For a better understanding of the changes in the integument or exoskeleton during the molt cycle and the terminology used in the next section, a brief explanation is given here. The lobster integument consists of 3 layers, the basement membrane, the epidermis and the cuticle. The cuticle is divided in four distinct layers, whereas the terminology varies, but the most widely used is that of Richards (1951): epicuticle, exocuticle, endocuticle and membranous layer see fig. 1 (Waddy *et al.*, 1995).

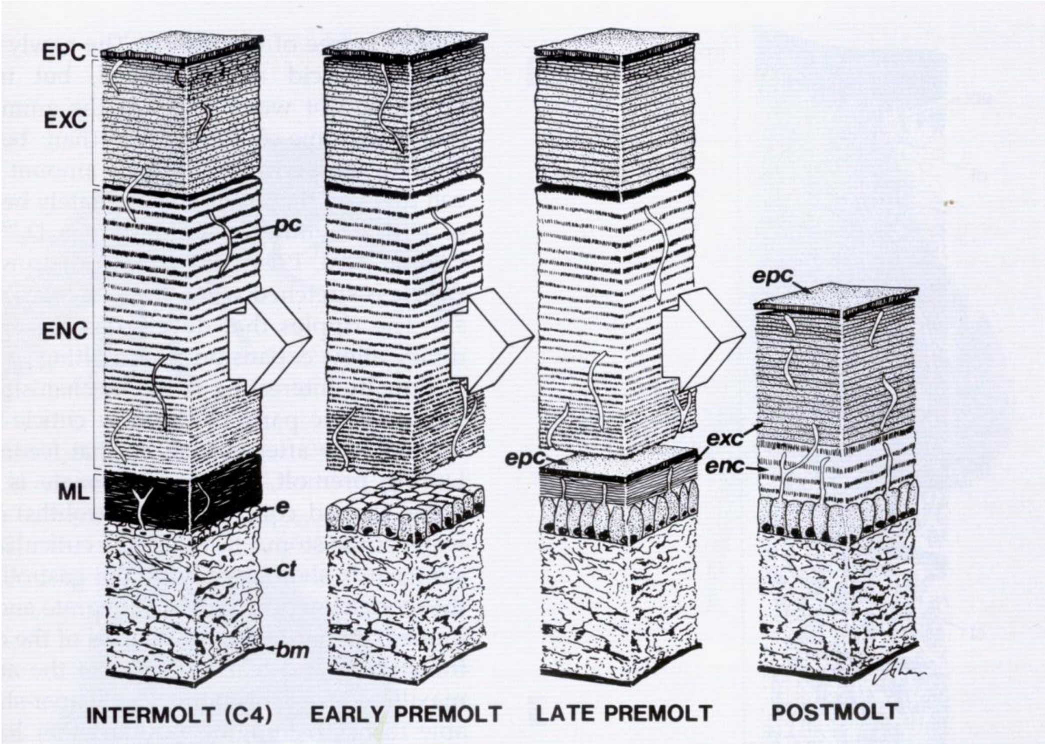

Fig. 1. Structural changes in the integument over the molt cycle. EPC, epicuticle; EXC, exocuticle; ENC, endocuticle; e, epidermis; ct, connective tissue; bm, basement membrane; pc, pore canals. (Waddy *et al*., 1995)

Stage E. Ecdysis, or shedding of the exoskeleton. A lobster is either preparing or recovering from this event. A lobster is capable of maintaining normal mobility, agility and awareness of its environment during pre-molt and even during the passive phase of the ecdysis. A loss in mobility only occurs during the active phase of ecdysis this usually lasts for 15-20 minutes (Aiken, 1980). The passive phase of ecdysis, also revered as stage D_4, consists of water absorption. The uptake begins approximately 1 hour before ecdysis and ends 2 hours after (Donald & Mykles, 1980). In the active phase the actual shedding of the old carapace begins due to the increased hydrostatic pressure, the animal is still mobile and may become agitated when disturbed. Unfavorable conditions determine whether this phase continues or not, delay can last for several hours when conditions for molt are not favorable. Eventually the thoraco-abdominal ruptures; when this happens *Homarus* becomes immobile and rolls to its side (Fig. 2.1, 2.2), the process is now irreversible. The old carapace becomes loose and turns forward along the posterior edge, now the head is free to rise and the cephalic appendages can be withdrawn. When eventually the abdomen is withdrawn, the animal enters stage A of the molt cycle (Waddy *et al*., 1955; Aiken, 1980).

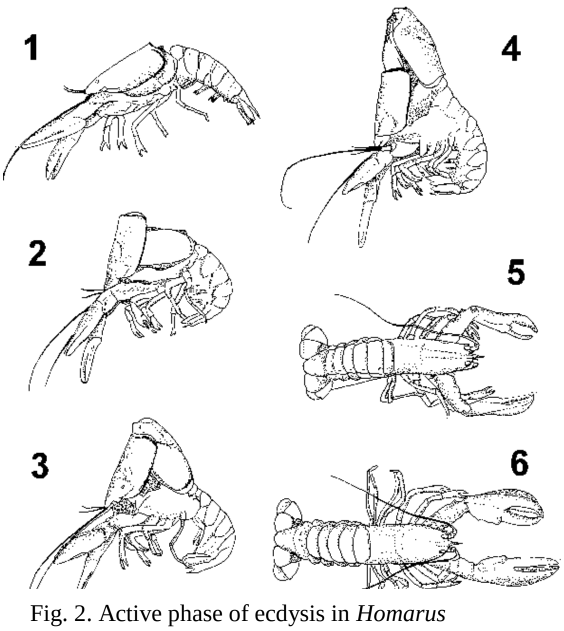

Fig. 2. Active phase of ecdysis in *Homarus americanus*. Thoracoabdominal membrane lifts clear of the new (1), and the lobster swings forward (2). The head appendages are withdrawn fist (3), then the thoracic appendages and finally the abdomen (4). (Aiken, 1980)

Stage A. Stage A is a very short stage and only occupies 2% of the molt cycle . Further absorption of water expands the animal to its new volume thereby the exoskeleton is soft and the animal does not feed. Growth is determined by the amount of cell division in the epidermis that occurs immediately before the onset of cuticle synthesis at molt stage D_1 and the degree to which the new cuticle is stretched after water absorption. (Waddy *et al., 1995; Travis, 1955).*

Stage B. The start of this stage is marked with appearing of the first endocuticle lamella, the formation continuous through stage C_3. The carapace becomes rigid in certain places as well. Therefore feeding does still not occur (Waddy *et al., 1995; Travis, 1955).*

Stage C. This stage is probably the longest of all stages. Stage C is divided in four sub stages, whereas stage C_3 is the end of post-molt. The integument has attained maximum rigidity at the end of stage C_3. In this stage the endocuticle synthesis is completed. Stage C_4 is also known as the inter-molt, at which the cuticle is completed including the presence of the membranous layer and the time when organic reserves are accumulating. (Waddy *et al*., 1955; Aiken, 1980).

Stage D. This stage represents the pre-molt or pro-ecdysis. In this period the lobster prepares for the next molt. In this stage the new exoskeleton is constructed under the old, while the old exoskeleton is gradually broken down (Waddy *et al*., 1995; Travis, 1955). The new skeleton only consists of the epicuticle and exocuticle, which is together the principal supportive layer prior to ecdysis (Aiken, 1980). The calcium ions that come free during the break down of the old skeleton, these ions are transported and accumulate to form gastroliths. They collapse in the stomach during ecdysis where they are digested. During post-molt remineralization of the new cuticle must occur and this relies on the digested calcium resource obtained from the gastroliths (Schechter *et al*., 2008).

Metamorphosis

A newly hatched lobster prelarva undergoes several molts before it settles on the ocean floor. The homarid lobster hatches as a pre larvae and then molts through 3 larval stages (I-III) see fig. 3. The different stages will be briefly discussed here.

Stage I is characterized by a segmented body with appendages, that are functional, on each segment. Large eyes can be observed and spines are visible on the rostrum and on the segments of the abdomen. Stage I larva have a telson as well. The In this stage the lobster larva is about 8mm long .

Stage II larva are a bit larger then stage I larva, they are about 9mm long. In this stage pleopods have developed and this is the main difference with the previous stage. These pleopods or "swimmerets" or located on the second through the fifth abdominal segment.

Stage III larva is in appearance more or less similar to the second stage, but they are again larger, approximately 11mm. They have a completed tail fan as well, with uropods and they possess larger claws (Ennis, 1995). Stages I-III are zoeal stages.

Stage IV is the post larval stage and metamorphosis has occurred during the molt from stage III to stage IV. The loss of larval characteristics like the dorsal abdominal spines and the formation of proportionally larger antennules and antennae makes the homarid lobster look very much alike an adult lobster, but the properties differ. The pleopods that were already developing in stage II are now functional. The

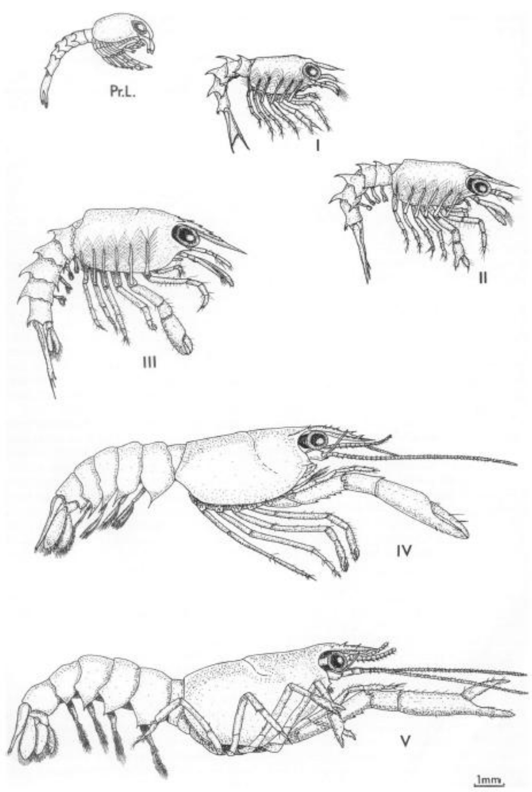

Fig. 3. Prelarva (Pr. L.), larvae (stages I, II, III), postlarva (stage IV), and stage V of *Homarus americanus* (Charmantier *et al*., 1991)

chelipeds of the first pereiopods are significantly larger than those of stage III. The transition from pelagic to benthic can now occur. Towards the end of stage IV a young lobster becomes progressively more benthic. This benthic behavior can be delayed when substrate quality and suitable shelter availability are not optimal (Ennis, 1995; Charmantier *et al*., 1991).
Stage V. In late stage V a lobster establishes on the ocean floor and benthic existence is now achieved. Increasing hydrostatic pressure induced upward movement in stage I-IV homarid lobster, but not anymore in stage V. The asymmetry in claws can be determined in this stage as well (Charmantier *et al*., 1991).

Endocrine control

The molt cycle is under control by endocrine processes, but this system is not yet fully understood (Phillips *et al*., 1980). The molt cycle was thought to be regulated by the interaction of two substances: molt-inhibiting hormone (MIH), which is stored and synthesized in the X-organ sinus gland complex of the eyestalk and molting hormone (MH), is most likely produced by a molting gland which is also referred to as the Y-organ located in the thorax. This theory is also known as the classical hypothesis. There may be a molt acceleration substance somewhere located in the central nervous system, but the classical hypothesis (two antagonistic hormones) remained the most popular hypothesis during 1960-1980 (Aiken, 1980).

The existence of a molt inhibiting hormone (MIH) was first assumed due to experiments with bilateral eyestalk ablation. Studies have shown that ablation of the eyestalk showed an acceleration in the molt cycle (Quackenbush & Herrenkind, 1980). Removal of the eyestalk during the inter-molt period usually results in the initiation of the process of pre-molt and eventually causes the animal to molt (Chaplin, 1973). The classical hypothesis or classical scheme, states that MIH acts directly on the molt gland to prevent synthesis and release of molting hormone. Later studies suggested that MIH controls the molt cycle by preventing tissues from reacting to MH. During this time one thought that pro-ecdysis is induced when the endocrine balance shifts towards the molting hormone. There has been done a lot of research regarding the endocrine control and there is evidence that the concept of the role of MIH should be modified (Aiken, 1980). More recent studies have actually indentified a molt inhibiting hormone. According to Chang *et al* (1993) MIH inhibits the synthesis and/or secretion of ecdysone in the Y-organ.

Molt induction occurs in decapods in general when the animal is in stage C_4 or inter-molt and enters stage D_0. The term “molting hormone” is a general term for whatever substance(s) cause premolt initiation and development. The two substances ecdysone and ecdysterone are probably involved in the normal molting cycle of a homarid lobster. MH is in general applicable to ecdysone and ecdysterone. There are results that indicate that ecdysterone is primarily responsible for epidermal and cuticular changes of mid and later pre-molt. These results also suggest that pre-molt induction is accomplished by something other than ecdysterone, this could be ecdysone. According to the classical hypothesis a crustacean enters pre-molt after eye-stalk removal because the molting gland produces molting hormone as soon as it is released from the inhibitory influence of the eyestalks’ derived MIH (Aiken, 1980). According to the research of Chang (1995) the substance that was previously revered to as ecdysterone is now indentified as 20-hydroxyecdysone (20-HE).

But… There is evidence for another substance, which is located somewhere other than the eye-stalk or the molting gland. This substance mediates some early premolt metabolic changes. The thoughts about the classical hypothesis are converging, because other research has been done, with conflicting results (Aiken, 1980).

With the results of later research the hormonal control can be summarized as follows: the molting gland or Y-organ secretes ecdysone and other ecdysteroids (molting hormones). Ecdysone is rapidly hydroxylated by several tissues to 20-HE. The role of 20-HE is the formation of the cuticle and it plays a part in the events in mid and late pre-molt. The hydroxylation of 20-HE will stop at an appropriate point, this is under the control of 20-HE as well. The role of MIH was rather speculative, but recent studies found that it does indeed inhibits the synthesis and/or secretion of ecdysone (Aiken, 1980; Chang, 1985; Chang *et al*., 1993).

Influencing factors

Factors like temperature, season and dominance order have a major impact on the molt cycle. The following part contains a brief description of these factors. Obviously there are many other influencing factors, but they are not discussed here, because they affect molt in a more general and obvious way and are not relevant for answering the main question.

Temperature. An elevated temperature may accelerates the growth rate, because the metabolism of a lobster is directly affected by the temperature, because a lobster is poikilotherm (relies on environmental temperature) and is proportional within the range of approximately 8 ^{o}C to 25 ^{o}C (Waddy *et al*., 1995). Molting rate is optimal between the temperatures of 15 ^{o}C and 20 ^{o}C. Molt rate declines above 20 ^{o}C and seldom occurs below 10 ^{o}C (Hughes & Matthiesen, 1962). This range thus suggests that a higher temperature gives a lobster a higher growth rate, then when they live in colder areas. The time and frequency is also influenced by the temperature. Lobsters that live in warmer areas like inshore areas grow faster and mature faster, which is not necessarily a good thing. Lobsters in warm areas molt 2 times a year, whereas lobsters that live in colder areas only molt during one molt peak in the late summer. When the temperature drops below 5 ^{o}C, molt induction is blocked. Such low temperatures may cause the intermolt lobster to stay in this state for 2 years.

Season. Molting occurs almost only during seasons when temperatures are optimal for growth. Aiken & Waddy (1976) investigated growth control and found that lobsters that were exposed to temperatures of 10 ^{o}C or higher in the spring, quickly entered pre-molt and ecdysis. Lobsters that were exposed to the same temperature but not during spring didn't molt. Temperatures between 15 and 20 ^{o}C, results in molting at any time of the year, illustrating that high enough temperatures thus override the seasonal molt inhibition. The "season awareness" is regulated by the neuro-secretory centers of the eyestalk (Waddy *et al*., 1995).

Dominance. Dominance is a factor that is effected by the molt cycle but it has an effect on the molt cycle as well. Both matters are covered here. Behavioral and social pressures influence molting and growth. It has been observed that the dominant lobster in a certain area always molts first. Subordinates suffer from this molt order in a way that they have a reduced increment and an extended intermolt period. The dominant lobster shows greater growth than would normally be attained in isolation (Aiken, 1980). Male dominance is correlated with the molt stage in a way that lobsters in early to middle pro-ecdysis (stages D_1 and D_2) are dominant over inter-molt animals (stage C), whereas late pro-ecdysis (D_3) and post-ecdysis (state A or B) animals are subordinate to those in inter-molt (Tamm & Cobb, 1978). When a dominant male molts he loses his dominant status, but this is regained a few days afterwards, when the integument has hardened (Cobb & Tamm, 1974).

Behavior

This section contains the alterations in behavior during the molt cycle. There are multiple changes in behavior like aggression, escape behavior and mating, these factors will be discussed separately. The mechanisms discussed in the previous section are now coming in hand for a better understanding of the behavioral changes, covered in the following section

Aggression

It is not surprising that a change in aggressive behavior may occur during different stages in the molt cycle. When a lobster is almost ready for molting (stage D_3), the old exoskeleton is beginning to break down or decalcifying. After molting (stage A and B) the new integument is still soft, calcification is not completed yet. These stages make the lobster vulnerable for attack and predation (Tamm & Cobb, 1978). Lobsters start to barricade their shelters up to two weeks (stage D_3) before molting and thus try to avoid contest, which consequently results in a decline of aggression. Aggressive behavior is low during the first to several weeks after molting. There has been observed a peak in aggression just before the vulnerable period in the molt cycle. These high levels of aggression may instill a “fear” in opponents, this can last during the vulnerable period, when an opponent could actually kill it former subordinate. This is probably an effect of prior agonistic experience. There seems to be some sort of memory of previous encounters and the outcome is carried over in to the next one (Atema & Cobb, 1980; Atema & Voigt, 1995). Securing and defending a shelter or an alternative shelter can be a reason for this sudden high level of aggression in stage D_1 and D_2 as well. (Atema & Voigt, 1995; Atema & Cobb, 1980).

Escape response

Regular tail-flipping occurs directly before and after ecdysis, stage D_3-C_1. The homarid lobster is most vulnerable at this time and shows submissive behavior and retreated or tail-flipped away when approached by non-aggressive conspecifics. Lobsters react to non-aggressive conspecifics with a higher frequency of tail-flips. This behavior was not observed during other stages of the molt cycle, this indicates a modification of responsiveness in reaction to conspecifics during the time surrounding ecdysis. This altered response is expressed in the avoidance of physical contact (Lipicus & Hernkind, 1982).

Factors like frequency, distance traveled, duration and sustained velocity, vary over the molt cycle and are studied in the American lobster. Pre-molt lobsters produce fewer tail-flips then soft-shelled, post-molt, lobsters do. Pre-molt lobsters travel for a shorter distance during a tail-flip and there velocity is less sustained that that of post-molt lobsters. Recently molted lobsters, (stage A), have a higher activity and a higher swimming frequency or tail-flips produced as well. Post-molt lobsters thus have a more sustained velocity during consequent tail-flips and they produce more tail-flips. These factors tend to compensate for a slower initial tail-flip (first flip of multiple consequent tail-flips) and acceleration of subsequent tail-flips. The slower initial tail-flip of recently molted lobsters is probably due to the muscle composition, which alters over the molt cycle. Recently molted lobsters can produce less force because there tissue is till absorbing water and muscle proteins are not yet fully synthesized (Cromarty *et al.,* 1987; Skinner, 1962; Skinner, 2005).

Hard-shelled, pre-molt lobsters probably react to threats with more aggressive behavior then escape behavior (Tamm & Cobb). These lobsters have a forceful initial tail-flip, though the following subsequent flips decrease in velocity and acceleration. These observations are applied on inter-molt lobsters as well. Their first flip is not as forceful as that of pre-molt lobsters, but the force of the first half of the following subsequent flips results in a not significantly difference in respect to work (Cromarty *et al*., 1987).

It is not yet clear which escape response is more effective, fewer tail-flips but with more force or more tail-flips with a more sustained velocity. Therefore it is quite possible that the difference is due to a difference in physiological condition. Soft-shelled lobsters prefer to escape rather than to fight and hard-shelled lobster give the favor to attack and perform a tail-flip with higher expenditure but they therefore don't perform a tail-flip that often then soft-shelled lobsters do (Cromarty *et al*., 1987).

Mating

A few days before a female homarid lobster molts, she leaves her shelter and pairs up with a male. During the hours before molting she repeatedly touches the male, which is described as "knighting". 30 minutes after the female completes her molt, the actual mating occurs. This happens either inside or near the male's shelter. The recently mated male and female stay together a few more days after mating. This period lasts for about 5 days and the male guards and protects the post-molt female. This can be to insure a female does not get attacked during her vulnerable period and thus possible loss of the male's genetic heritage. Excluding other mating partners is also accomplished in this way (Aiken & Waddy, 1980; Atem, 1986). Templeman (1934) stated that homarid lobsters where only able to mate when the female had a very soft shell. There was no success in a promoted mating act between hard-shelled males and females that had molted 2-3 days ago. Therefore mating after molt stage A or B was considered unusual (Aiken & Waddy, 1980). However later studies have shown that inter-molt mating can occur. This study argued that mating is not dependent on the molt stage of the female, however there was a peak in female receptivity immediately after molting (Dunham & Skinner-Jacobs, 1978). There are speculations that inter-molt matings are useful for females that are nonsocial and or widely dispersed across the sea bottom. This would allow the female to collect sperm at any time she happened to cross paths with a mature male (Atema, 1986). According to the observations of Templeman (1934), male lobsters where not able to successfully mate in all different molt stages. Soft-shelled males have more difficulties with turning the female over during the mating act (Templeman, 1934). This suggests that male lobsters are reliant on their molt stage. Females visit only shelters of dominant males to find a partner; they take the initiative. This would suggest that it wouldn't be smart for males to molt during the mate season, because they lose their dominant status for a month or more when they molt, when molting a male can be evicted by a previous subordinate. When a male is not capable of occupying a proper shelter and find a mating partner, he should actually molt. This strategy gives him the chance to outcompete the currently successful males, because it has increased in size.

Discussion

In mechanisms section the general aspects of the molt cycle were covered first. This was meant to give insight in whatever motivates a lobster to the specific and distinct behavior during the molt cycle. The second section contained descriptions of the differences in behavior during the molt cycle. There, aspects like aggression, escape behavior and the influence of the molt cycle on mating were described. My research question was; what are the internal changes during the molt cycle and how do they alter the homarid lobster's behavior? The next part gives a summary of my findings and should answer this question.

The molt cycle is divided in 5 stages and several sub stages. Four basic periods in the molt cycle cover these stages. The first period is the molt and this stage covers stage E, whereas stage D_4 is also said to be the passive phase of ecdysis. This stage describes the actual molt, the shedding of the old exoskeleton. The second period is referred to as the post-molt; this period encompasses stage A, B and C_1 through C_3. In the stages A and B the lobster's exoskeleton is still very soft, he is recovering from molt. A lobster is not eating in these stages yet. At the end of stage C_3 the integument has full rigidity and the lobster is recovered now from ecdysis. The third period, or inter-molt, encompasses stage C_4 and this is the time when reserves are accumulating. The last stage before the next ecdysis is pre-molt, or stage D. In this period the lobster is preparing for the upcoming molt. A new exoskeleton is formed under the old one and the old integument is gradually broken down.

A major event in a homarid lobster's life cycle is the metamorphosis in pre larval stage III to post larval stage IV. When the molt from stage III to stage IV has occurred a lobster is called a juvenile, in this stage a homarid lobster is ready to settle on the ocean floor and exchange his pelagic lifestyle for a benthic existence.

There has been a lot of research regarding the endocrine control of molt on the Homarid lobster, but it is not yet fully understood. The current hypothesis states that the molt cycle is under control of a molting hormone (MH). This hormone is secreted and stored in the molting gland or Y-organ. The secreted hormone is ecdysone and it causes apolysis at the start of gastrolith formation and it induces activity in the ecdysone 20-monoxygense system. With this system the rate of ecdysterone formation is induced. Ecdysterone has influence on the cuticle formation and other events during mid and late premolt. The role of a molt inhibiting hormone (MIH) which is presumably stored and synthesized in the X-organ sinus gland complex of the eyestalk is more speculative. According to recent studies this hormone has a direct effect on the secretion or synthesis of ecdysone.

Temperature hast a great influence on the molt cycle. It determines whether molt will occur or not. Ecdysis can even be delayed when the temperatures are not optimal. Seasonal variety has influence as well, bot temperature and seasonal variations determine whether a lobster molts or not. The dominance order is of importance for the molt cycle as well. Dominate homarid lobsters molt first and have an increased growth. Subordinate may even experience an increased inter-molt stage.

With all these mechanical aspects in mind it should be possible to explain some of the behavioral changes during the molt cycle. There have been some alterations in aggression and escape behavior observed over the molt cycle. Just before and after ecdysis a lobster is experiencing a vulnerable period. In this period a low level of aggressiveness is observed. This seems logical, because in this period (stage D, A and B) the exoskeleton is very soft and a homarid lobster tries to avoid contest.

During the time just before the vulnerable a peak in aggressiveness has been observed. The reason for this peak is probably to instill fear in opponents. When lobsters are in the vulnerable period, they could be attacked by their former subordinates, but because of the high aggressive period prior to the vulnerable period, they will hopefully not be encountered by their former subordinates. This peak in aggressiveness could be to ensure a shelter that will be occupied during the vulnerable period as well. When a lobster encounters a treat in the vulnerable period he will most likely perform tail-flips to avoid contest. This behavior declines as the exoskeleton hardens.

The molt cycle is of influence on mating as well. It is preferable for a female lobster to be in a stage shortly after ecdysis. During this stage receptivity for mating is at its highest and mating is more likely to occur. There has been recently data published which states that females can mate during stages in the molt cycle when the exoskeleton is already hardened. This could be a strategy for non-social females, who can collect sperm at any time, and thus aren't reliable on their state in respect to the molt cycle. The effect of the molt cycle on the male lobster are alterations in dominance order. A former dominant male loses its dominant state when it is molting. Females take the initiative in finding a partner and they choose for the dominant male. Molting during the pair season for a male would not be smart, because they have a lower change on copulating with a female.

Nevertheless there are some remarks to be made. They way metamorphosis has been described in this thesis seems to be acceptable, but there are researches that designated the so-called first post-larval stage or first juvenile to stage V. Since metamorphosis is for the greater part accomplished at the molt from stage III to stage IV, I give the preference to address stage IV as the first post-larval stage. This is in agreement with the opinion of Charmantier *et al* (1991). The reason to address the first juvenile stage to stage V could be that in this stage the actual settlement on the ocean floor occurs, whether settlement in stage IV is progressively occurring.

It appears in this thesis that endocrine control is not yet fully understood. Research is still in progress regarding this subject, which give new insights that may reject earlier views. Due to more recent research the composition of the molting and molt inhibiting hormones are discovered. This gives new insight regarding the role of MIH. Its role was rather speculative and it was thought that it had an effect on tissue response to ecdysone, but more recent studies have concluded that it directly effects the synthesis and/or secretion of ecdysone. Due to these more recent developments the endocrine control of the homarid lobster's molt cycle is described in more detail. It is most likely that the future brings more insight in the hormonal changes during molt and that research has to be continued to this matter.

In the past one thought that female lobsters are dependent on the stage in their molt cycle and that fertilization could only occur during the soft-shelled period just after ecdysis. It is partly true, because a peak receptivity for female lobster is just after ecdysis (Templeman, 1934; Aiken & Waddy, 1980). Later studies have shown that mating can occur during other, hard-shelled, stages of the female molt cycle (Dunham & Skinner-Jacobs, 1978). The possibility of inter-molt mating by the female lobster, could explain why a male provides shelter after the mating act. When mating occurs with a recently molted female it is obvious that a female is vulnerable for predation due to her soft integument. On the other hand due to the new insights regarding inter-molt mating, the provision of shelter by the male prevents the female from mating with other males. The Discovery of Waddy & Aiken (1986) reveals that older, female, American lobsters, who molt only ever other or third year can actually fertilize a batch of eggs each year with one insemination. This could be a strong indication for post-copulatory guarding as well. When a female is inseminated by only one male, results in a higher amount of offspring for the male, due to fertilization of eggs by his sperm in other years. One could say that this discovery removes the last doubts of the necessity of inter-molt mating. Apparently sperm can be stored and used for several years (Atema, 1986).

References

Aiken, D.E. 1980. Molting and growth. In: *The Biology and Management of Lobsters. Vol.1* (Ed. By J.S. Cobb & B.F. Philips), pp. 91-163. Academic Press, New York, NY.

Aiken, D.E. & Waddy, S.L. 1980. Reproductive biology. In: *The Biology and Management of Lobsters. Vol.1* (Ed. By J.S. Cobb & B.F. Philips), pp. 215-276. Academic Press, New York, NY.

Aiken, D.E. & Waddy, S.L. 1995. Aquaculture. In: *Biology of the Lobster Homarus americanus.* (Ed. By J.R. Factor), pp. 153-175. Academic Press, San Diego, Ca.

Atema, J. & Cobb, J.S. 1980. Social behavior. In: *The Biology and Management of Lobsters. Vol.1* (Ed. By J.S. Cobb & B.F. Philips), pp. 409-450. Academic Press, New York, NY.

Atema, J. 1986. Review of sexual selection and chemical communication in the lobster, *Homarus americanus*. *Can J. Fish. Aquat. Sci.* **43**: 2283-2390

Atema, J. & Voigt, R. 1995. Behavior and sensory biology. In: *Biology of the Lobster Homarus americanus.* (Ed. By J.R. Factor), pp. 313-348. Academic Press, San Diego, Ca.

Ayers, J., Zavracky, P., McGruer, N., Massa, D., Vorus, V., Mukherjee, R. & Currie, S. 1998 A Modular Behavioral-Based Architecture for Biomimetic Autonomous Underwater Robots. In: *Proc. of the Autonomous Vehicles in Mine Countermeasures Symposium.* Naval Postgraduate School., In press.

Chang, E.S. 1985. Control of molting in decapod crustacea. American zoologist **25**: 176-185

Chang, E.S., Bruce, M.J. & Tamone, S.L. 1993. Regulation of crustacean molting: A multi-hormonal system. *Amer. Zool* **33**: 324-329

Chaplin, J.M. 1973. Aspects of growth in the New Zealand rock lobster *Jasus edwardsii*. MSc thesis.

Charmantier, M., Charmantier-Daures, M. & Aiken, D.E. 1991. Metamorphosis in the lobster *Homarus* (Decapoda): A Review. Journal of Crustacean Biology **11**: 481-495

Cromarty, S.I., Cobb, J.S. & Kass-Simon, G. 1991. Behavioral analysis of the escape response in the juvenile lobster *Homarus americanus*. *J. exp. Bio* **158**: 565-581

Cobb, J.S. & Philips B.F. 1980. Preface. In: *The Biology and Management of Lobsters. Vol.1* (Ed. By J.S. Cobb & B.F. Philips), pp. xi-xiii. Academic Press, New York, NY.

Cobb, J.S. & Tamm, G.R. 1974. Dominance status and molt order in lobsters (*Homarus americanus).* Marine behavior and physiology **3**: 119-124

Donald, L. & Mykles, L. 1980. The mechanism of fluid absorption at ecdysis in the American lobster, *Homarus americanus*. *J. exp. Bio* **84**: 89-101

Ennis, P.S. 1995. Larval and Post larval Ecology. In: *Biology of the Lobster Homarus americanus.* (Ed. By J.R. Factor), pp. 23-46. Academic Press, San Diego, Ca.

Dunham, P.J. & Skinner-Jacobs, D. 1978. Intermolt mating in the lobster *(Homarus americanus)*. Marine behavior and physiology **5**: 209-214

Helluy, S.M. & Beltz, B.S. 1991. Embryonic development of the American lobster (*Homarus americanus*): Quantitative staging and characterization of an embryonic molt cycle. *Biol. Bull.* **180**: 355-371

Hughes, J.T. & Matthiessen, G.C. 1962. Observations on the biology of the American lobster, *Homarus americanus*. Limnology and oceanography **7**: 414-421

Lipcius, R.N. & Hernkind, W.F. 1982. Molt cycle alterations in behavior, feeding and diel rhythms of a decapod crustacean, the spiny lobster *Panulirus argus*. Marine biology **68**: 241-252

Phillips, B.F., Cobb, J.S. & George, R.W. 1980. General Biology. In: *The Biology and Management of Lobsters. Vol.1* (Ed. By J.S. Cobb & B.F. Philips), pp. 1-82. Academic Press, New York, NY.

Quackenbush, L.S. & Herrenkind, W.F. 1980. Regulation of molt and gonadal development in the spiny lobster, *Panulirus argus* (Crustacea: palinuridae): effect of eyestalk ablation. Comparative biochemistry and physiology part A: physiology **69**: 523-527

Schechter, A., Berman, A., Singer, A., Freiman. A, Grinstein, M., Erez, J., Aflalo, E.D. & Sagi, A. 2008. Reciprocal changes in calcification of the gastrolith and cuticle during the molt cycle of the red claw crayfish *Cherax quadricarinatus. Biol bull.* **214**: 112-134

Skinner, D.M. 1962. The structure and metabolism of a crustacean integumentary tissue during a molt cycle. *Biol bull*. **123**: 635-647

Skinner, D.M. 2005. Breakdown and reformation of somatic muscle during the molt cycle of the land crab, *Gecarcinus laterallis*. Journal of experimental zoology **163**: 115-123

Tamm, G.R. & Cobb, J.S. 1978. Behavior and the crustacean molt cycle: Changes in aggression of *Homarus americanus*. Science **200**: 97-81

Templeman, W. 1934. Mating in the lobster. Contributions to Canadian biology and fisheries **8**: 421-432

Travis, F.D. 1955. The molting cycle of the Spiny lobster, *Panulirus arguslatreille*. II. Pre-ecdysial histological and histochemical changes in the hepatopancreas and integumental tissues. *Biol. Bull* **108**: 88-112

Waddy, S.l. & Aiken, D.E. 1986. Multiple fertilization and consecutive spawning in large American lobster, *Homarus americanus*. *Can. J. Fish. Aquat. Sci*. **43**: 2291-2294

Waddy, S.L., Aiken, D.E. & Kleijn De, D.P.V. 1995. Control of Growth and Reproduction. In: *Biology of the Lobster Homarus americanus.* (Ed. By J.R. Factor), pp. 217-266. Academic Press, San Diego, Ca.