RECUPERATION OF *Nodipecten nodosus* SCALLOP SPATS (LINNAEUS, 1758) AFTER DIFFERENT PERIODS OF PERMANENCE IN LABORATORY AND IN THE SEA

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SUMMARY

The spats recuperation rate of *Nodipecten nodosus* scallop was analyzed in three different settlement times, in the laboratory and with respect to permanence in the sea. Two experiments were carried out in the Santa Catarina region, South of Brazil: the first from August to October of 2000 and the second from March to May of 2001. The eyed larvae obtained from the LMM-UFSC, were placed to be settled in collectors and remained in the laboratory for 15, 25 and 35 days. When transferred to the sea the collectors remained in the sea 10, 20 and 30 days. Regarding the recuperation in the laboratory, the highest value was obtained after 15 days of settlement in both experiments 1 and 2 (1.34 % and 1.30 % respectively). For recuperation of the spats in the sea there was no significant difference between the different times of permanence. However, when analyzing and comparing results of both the recuperation in the laboratory and that carried out in the sea, it was observed that there is a significant decrease in the recuperation of the animals in the different permanence times in the sea for spats with 15 days in the laboratory. For spats with 20 and 30 days of permanence in the sea, there was no significant difference in the recuperation rate they remained 15, 20 and 35 days in the laboratory. This study shows that for the recuperation of spats the best results were those which remained in the laboratory for 15 to 25 days and in the sea for 20 days.

Key words: *Nodipecten nodosus*; Recuperation; Spats

RECUPERAÇÃO DE PRÉ-SEMENTES DA VIEIRA *Nodipecten nodosus* (LINNAEUS, 1758) APÓS DIFERENTES PERÍODOS DE PERMANÊNCIA NO LABORATÓRIO E NO MAR

RESUMO

A recuperação de pré-sementes da vieira *Nodipecten nodosus* foi analisada em três diferentes tempos de assentamento em laboratório e de permanência no mar. Foram realizados dois experimentos, sendo o primeiro de agosto a outubro de 2000 e o segundo experimento de março a maio de 2001 na região de Santa Catarina/Brasil. No mar, os experimentos foram realizados no município de Porto Belo/SC-Brasil. As larvas "olhadas" obtidas no LMM-UFSC foram colocadas para assentamento e permaneceram no laboratório durante 15, 25 e 35 dias. Após cada período de assentamento, as pré-sementes foram transferidas para o mar, nos coletores dentro de bolsas, as quais permaneceram no mar 10, 20 e 30 dias. Para a recuperação em laboratório o maior valor foi obtido para 15 dias de assentamento nos experimentos 1 e 2 (1,34% e 1,30 % respectivamente). Na recuperação das pré-sementes do mar não houve diferença significativa entre os diferentes tempos de permanência. Entretanto, com uma análise da interação dos resultados de recuperação do laboratório e do mar, observou-se que para pré-sementes com 15 dias de laboratório há uma diminuição significativa na recuperação dos animais nos diferentes tempos de permanência no mar. Para pré-sementes com 20 e 30 dias de permanência no mar não houve diferença significativa se estas permaneceram 15, 25 ou 35 dias no laboratório. Com a realização deste estudo, pôde-se concluir que os melhores resultados de recuperação de pré-sementes foram para aquelas que permaneceram 15 a 25 dias no laboratório e 20 dias no mar.

Palavras chave: *Nodipecten nodosus*; Recuperação; pré-sementes

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INTRODUCTION

The main critical point in the cultivation of pectinids, as well as other marine mollusks, is the obtainment of larvae and seeds (AVENDAÑO and CANTILLANEZ, 1989; HARDY, 1991). Research on the obtainment of *Nodipecten nodosus* seeds through artificial collectors placed in a natural environment, demonstrated that this species had a low collection rate (OSTINI and POLI, 1990; MANZONI e RUPP, 1993; MANZONI e POLI, 1996). LOOSANOFF and DAVIS (1963) developed techniques which benefited the production of bivalve mollusk larvae in laboratory. Currently, specific techniques are used for pectinids, such as those of CHEW *et al.* (1987), BOURNE *et al.* (1989) and ILLANES (1990).

According to URIBE (1989), for *Argopecten purpuratus* the survival of eyed larvae, up to spats with 2.5 mm, is around 12% to 20%. The settlement and spats phases are also critical stages for the cultivation of bivalves (SASTRY, 1965; BOURNE *et al.*, 1989), in particular for species with little tradition in larvae cultivation such as *Nodipecten nodosus*. In the settlement, the mortality of larvae can be due to the substratum and the sensibility to changes in the physical and chemical parameters of the environment (YAMAMOTO, 1964).

After the settlement phase, the animals denominated as spats or young can be transferred to the sea or remain in the laboratory for some time (BOURNE and HODGSON, 1991). It is important to highlight, however, that in these phases the animals are very vulnerable to various factors such as, dehydration, predation, suffocation, or any other environmental stresses (LAING, 2002). Therefore, the way the animals are transported to the sea can affect their survival. Guaranteeing sufficient water and adequate and constant temperature are procedures that can diminish stress during transportation. In addition, the maintenance of the structures at the cultivation locale, from the removal of the incrusted organisms to the periodic inspection of the sustaining system can guarantee a higher chance of survival in this phase (LAING, 2002).

The studies about aspects of larvae and settlement in *Nodipecten nodosus* have been developed in southern Brazil since 1994, and included in many experiments like RUPP (1994), RUPP and POLI (1994), RUPP (1996), RUPP and PARSONS (2004), RUPP *et al.* (2004a), RUPP *et al.* (2004b), RUPP *et al.* (2005) and ZANETTI (2007). However, none of them establishes solve the decision of how many times we have to keep the larvae in laboratory and in the sea after detachment to improve the survival and grow.

Therefore, with the objective of contributing to the cultivation techniques in the settlement phase of *Nodipecten nodosus*, and to the viability of the commercial production of this pectinid, this study analyzed the recuperation, in the settlement phase, of *Nodipecten nodosus* spats after different time periods in laboratory and in the sea

MATERIAL AND METHODS

The experiment for the study of spats recuperation in laboratory settlements was carried out in the Laboratório de Moluscos Marinhos (Marine Mollusk Laboratory) (LMM), at the Federal University of Santa Catarina (UFSC), located in Florianopolis/SC-Brazil. A surface long line was used to maintain the spats and the collectors in the sea, located in the municipality of Porto Belo/SC-Brazil (5 meters deep).

Egg laying inducement / larvae culture

The reproducers of *Nodipecten nodosus* were maintained in the laboratory, in tanks with circulating sea water, at a temperature of 17 ^{0}C and with constant ventilation, daily feeding constituted of micro-algae *Isochrysis galbana* variety Tahiti and *Chaetoceros calcitrans*, to a final concentration of 2 x 10^4 cel.mL^{-1}, remaining at least 15 days in this system before egg laying inducement.

The LMM used the technique for egg laying inducement and fertilization according to RUPP (1994) and RUPP (1996), with high phytoplankton density and water temperature variation (from 17 ^{0}C to 26 ^{0}C) in the inducement tanks. In the larval cultivation the techniques RUPP (1994) and RUPP *et al.* (1997) were also used, which basically consist of: transference of the embryos to the larvae-culture tanks; daily tank water changes; filtering with different meshes; daily feeding with micro-algae (*Isochrysis galbana* variety Tahiti, *Chaetoceros calcitrans* and *Nannochloropsis oculata*); antibiotic (Chloranphenicol) addition in the food and controlled water temperature, varying from 23 ^{0}C to 25 ^{0}C.

Experiment 1: Settlement carried out between August and October, 2000

In each settlement tank containing settling stage larvae, with a volume of 100 liters, 45 polypropylene collectors were placed as substratum for larvae

settlement. In this experiment, nine tanks were used. In each tank 200,000 settling stage larvae were placed (an approximate density of 1.9 larvae.mL^{-1}), totalizing 1,800,000 individuals. The daily food of the larvae was constituted of a combination of three micro-algae: *Isochrysis galbana*, *Chaetoceros calcitrans* and *Nannohcloropsis oculata*, in final concentrations of 4,000 to 5,000 cells.mL^{-1} in the settlement tanks.

Experiment 2: Settlement carried out between March and May, 2001

In each settlement tank containing settling stage larvae, with a volume of 300 liters, 48 polypropylene collectors were placed as substratum for larvae settlement. In this experiment 3 tanks were used. In each tank 400,000 settling stage larvae were placed (an approximate density of 1.3 larvae.mL^{-1}), totalizing 1,200,000 individuals. The daily food of the larvae was constituted of a combination of three algae: *Isochrysis galbana*, *Chaetoceros calcitrans* and *Nannochloropsis oculata*, in final concentrations of 3,000 to 5,000 cells.mL^{-1} in the settlement tanks.

In both experiments, the water temperature was maintained at 25 ± 0.5 ^{0}C, the ventilation was constant, the tank water changes were carried out daily and the spats remained under settlement conditions, in the laboratory for three time periods: 15 days; 25 days and 35 days. To minimize the bacterial growth during the settlement process in laboratory, an antibiotic (Chloramphenicol) was added to the micro-algae, in a concentration of 5 mg.L^{-1}. At the end of each settlement time in the laboratory, an allotment was transferred to the sea, represented by nine nitex mesh bags, with mesh aperture of 500 µm, in each of which four collectors from the settlement tanks were placed. Each transferred allotment remained in the sea for three different time periods: 10 days; 20 days and 30 days, in both experiments. For each time period they remained in the sea, triple samples were used.

Samplings

Before transferring each allotment to the sea an initial sampling was carried out to quantify the spats settled in the collectors. The sampling number was three collectors/tank for experiment 1, and four collectors/tank for experiment 2. Using a brush, the seeds were detached from the collectors and a total count was carried out for each of the collectors. In the sea, the collectors were placed inside the bags and suspense in surface long lines at depths of two meters. The bags were brushed once a week to remove incrusting organisms to maintain the structures.

In each allotment, three bags of each permanence time in the sea were returned for sampling. In the laboratory, the samplings were carried out with a brush. After removing the collectors, all the spats were counted with the help of a microscope and/or magnifying glass.

RESULTS

Settlement of spats in laboratory

Both in experiment 1 and 2, the number of spats recuperated in laboratory from the quantity of settling stage larvae placed for settlement, showed significant differences ($p < 5\%$) among the different times of settlement, after the multiple variance analysis and comparison test among measurements, according to the test Tukey (HSD).

The best result in experiment 1 was obtained after 15 days of settlement in laboratory with an average recuperation of 178.7 individuals per collector (Table 1) ($F = 15.98129$; < 0.0001; $df = 2$). In experiment 2, the best result was also obtained after 15 days of settlement in laboratory with an average recuperation of 108 individuals per collector (Table 1) ($F = 4.357312$. $\alpha < 0.0209$; $df = 2$). In experiment 1, the standard deviation of the results presented very high values; in experiment 2, these values were lower.

Recuperation of spats from the sea

According to the results obtained in the recuperation of spats from the sea, neither experiment 1 (Table 2) nor experiment 2 (Table 2) showed significant differences ($p > 5\%$) among the different times of settlement in the laboratory that were returned to the sea, after a non-parametric analysis of the data using the Kruskall-Wallis test.

Interaction analysis of the laboratory and sea effects

With the objective of analyzing the interaction between the recuperation of spats in laboratory and in the sea, the multiplication of spats recuperation percentage in laboratory by the spats recuperation percentage in the sea, was carried out for all time periods tested.

When these two effects are analyzed together ($p = 5\%$), in both experiments 1 and 2, a significant difference can be observed, after the Qui-square test, for spats after 15 days in laboratory. For experiment 1,

the highest recuperation value was obtained after 10 days in the sea, and the lowest value after 30 days in the sea, respectively, 13.89 and 4.51 (Figure 1A) (Q_2 = 4.789853; $p < 0.05$ and df = 1). For experiment 2, the highest recuperation value was obtained after 20 days in the sea, and the lowest value after 30 days, respectively 37.16 and 11.43 (Figure 2B) (Q_2 = 13.61912; $p < 0.01$ and df = 1). For samples with 25 and 30 days in laboratory there was no significant difference, after the Qui-square test, in the joined affect analysis, in both experiments 1 and 2 (Figure 1A and 1B).

Table 1. Average number of *N. nodosus* spats recovered per collector in the different time periods of settlement in laboratory, and survival percentage (N = 9) for experiments 1 and 2

Time of settlement (days)	*Average ± divergence*	*Survival (%)*
Experiment 1		
15	178.7 ± 91.59 [a]	1.34
25	60.8 ± 41.82 [b]	0.46
35	25.6 ± 26.50 [b]	0.19
Experiment 2		
15	108.0 ± 39.43 [a]	1.30
25	59.9 ± 14.43 [b]	0.72
35	51.4 ± 16.06 [b]	0.62

Table 2. Recuperation of *N. nodosus* spats from the sea, for each settlement time in the laboratory (N = 3) for experiments 1 and 2, expressed in percentage

Time of permanence in the sea (days)	*15 days of settlement in laboratory (%)*	*25 days of settlement in laboratory (%)*	*35 days of settlement in laboratory (%)*
Experiment 1			
10	10.35 ± 4.75	23.05 ± 11.31	16.67 ± 14.23
20	8.11 ± 3.82	11.52 ± 5.50	12.75 ± 9.74
30	3.36 ± 1.65	12.89 ± 9.83	9.48 ± 5.08
Experiment 2			
10	18.72 ± 9.72	39.44 ± 14.26	38.31 ± 16.44
20	28.09 ± 16.45	32.04 ± 7.61	27.49 ± 6.33
30	8.54 ± 4.84	32.02 ± 3,02	23.59 ± 8.57

Also, a significant difference was observed after the Qui-square test, in the recuperation of spats that remained in the sea for 10 days after all the settlement times, 15, 25 and 35 days in the laboratory, in both experiments. For experiment 1, the highest recuperation time obtained was 13.89 for 15 days of laboratory, and the lowest 3.25 for 35 days (Figure 1A) (Q_2 = 6.608767. $p < 0.01$ and df = 1); and for experiment 2, the highest recuperation value was of 24.91 for 15 days of laboratory, the lowest being 6.84 for 35 days (Figure 1B) (Q_2 = 10.28489; $p < 0.01$ and df = 1).

In experiment 1, after 20 days in the sea, there was also a significant difference, after the Qui-square test, for the joined effect of spats that remained in the laboratory for 15 and 35 days, the highest value being 10.89 for 15 days, and the lowest 2.49 for 35 days (Figure 1A) (Q_2 = 5.281491. $p < 0.05$ and df = 1).

In experiment 2, after 20 days in the sea, the significant difference obtained after the Qui-square test, was for the three time periods 15, 25 and 35 days of settlement in laboratory, the highest value being 37.16 for 15 days and the lowest 4.87 for 35 days (Figure 1B) 1 (Q_2 = 24.80943; $p < 0.01$ and df = 1).

For the effect of spats that remained in the sea for 30 days, there was no significant difference, after the Qui-square test, in the settlement times tested in laboratory for both experiments 1 and 2 (Figure 1A and 1B).

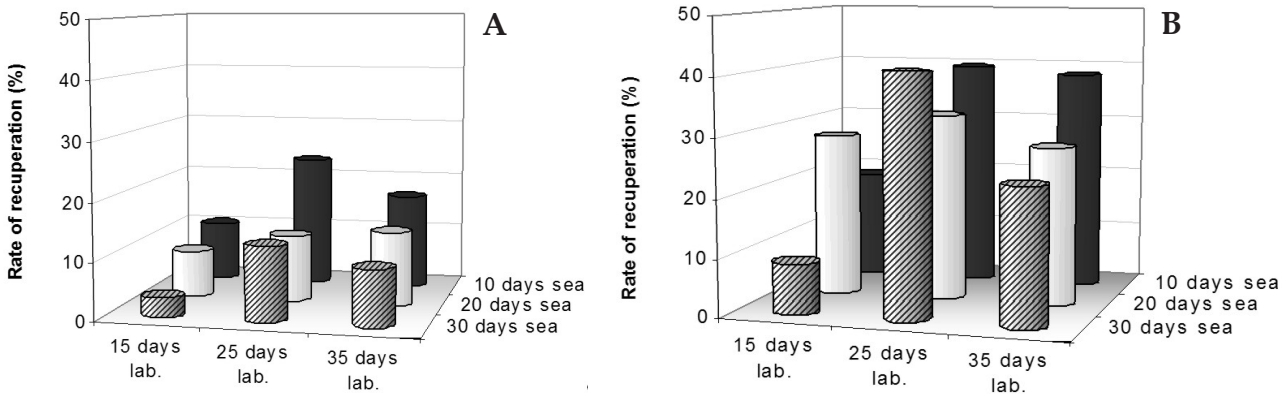

Figure 1. Analysis of the joined effect of recuperation percentages of *Nodipecten nodosus* spats maintained for different time periods in the laboratory and in the sea; A) for experiment 1; and B) for experiment 2

DISCUSSION

In this study, the minimum period of permanence (15 days) in laboratory was tested of the larvae of *Nodipecten nodosus* to guarantee a higher percentage of their metamorphosis. According to SASTRY (1965), and HODGSON and BOURNE (1988), the development period of pectinid larvae depends mainly on the species cultivated and the temperature.

According to SASTRY (1965), the metamorphosis process does not occur instantaneously in the settlement phase. URIBE (1989), for *Argopecten purpuratus*, mentions that this process generally is completed within 48 hours and that the settlement phase takes an average of 15 days. During this period, many larvae after settling, detach from the subtracts, float in water column until settling again later (SASTRY 1965; URIBE 1989).

Another factor that helped in determining the minimum time of 15 days was the size of the spats. According to URIBE (1989), the pectinid spats of *Argopecten purpuratus* reached an average size of 450 μm, after this settling time. The bags used in this experiment to transfer the collectors to the sea have a minimum mesh size of 500 μm, since a smaller mesh than this makes difficult the passage and circulation of water inside the bags.

The results of the present study showed that in both experiments 1 and 2, the recuperation of the *Nodipecten nodosus* spats after settling in laboratory is higher in the first 15 days of settlement, decreasing throughout days after that, with the lowest values recorded at 35 days of settlement.

In the phase of settlement and during metamorphosis, the survival of the pectinid larvae can be affected by the water temperature, salinity, cultivation density, quantity and quality of food, abundance of predators and by natural mortality occurring in this phase (YAMAMOTO, 1964) Also, the collector position and orientation (PEARCE and BOURGET 1996, HARVEY *et al*., 1997, TAYLOR *et al*., 1998, DE LA ROCHE *et al*., 2005), the water current, in relationship to the collector deep installation (HODGSON and BOURNE 1988, PEARCE *et al*., 1994, MANUEL *et al*., 2000, ROBERT and NICOLAS 2000) has been described to interfere in this phase survival .

In this study, the decrease in the spats average recuperation during the period in laboratory or in the sea can be related, among other factors, to the natural mortality of pectinids which occurs in this phase. This fact has also been described by PAUL *et al*. (1981) for *Chlamys opercularis* and *Pecten maximus*.

Many food sources can be found in the sea including, different species of microalgae and organic material in suspension. On the other hand, in the laboratory, the food sources are restricted to two, three, or in some cases four species of microalgae, which can affect the survival of the spats during this time. BOURNE and HODGSON (1991) mention that, for *Patinopecten yessoensis* in initial phase, a diet with three or four species of microalgae does not supply a complete nutritional diet. Not only the quality but also the amount of food can effect the spats survival as described by NICOLAS and ROBERT (2001).

Another important aspect in the quality and nutritional value of the food is the relationship between the presence of lipids and fatty acids (PUFAS) and larval survival, as described by MILKE *et al*. (2004) for *Placopecten magellanicus* . The density

of the larvae in the settlement is an important factor that influences the success of the metamorphosis. In the present study a density of 1.3 and 1.9 larvae mL^{-1} was which is close to that recommended for other species. As an example, according to BOURNE and HODGSON (1991), for *Patinopecten yessoensis* with a density of 2 larvae mL^{-1}, 81.5% of the metamorphosis was obtained.

When analyzing the interaction of recuperation percentages in the laboratory and in the sea, the joined effect, it can be observed that for spats with 35 days in laboratory, as well as those with 30 days in the sea, the recuperation in the different times tested did not vary. This probably occurs because the older spats are bigger, and more resistant to predators, suffocation, handling, fouling and variations in environmental conditions.

Referring to predators, AVENDAÑO and CANTILLANEZ (1989), attribute the loss of *Argopecten purpuratus* spats to the presence of crustaceans in the collectors. BRAND *et al*. (1980) also mention the decrease of *Chlamys opercularis* and *Pecten maximus* spats, in the sea, due to the presence of different species of predator crustaceans in the collectors. According to DISALVO *et al*. (1984), the larvae of predator crustaceans enter the bags and complete their development inside the bags, feeding on *Argopecten purpuratus* spats. This was proven by the authors above, through the presence of a great quantity of shells found at the bottom of the bags. Data showing the presence of predators were not collected in the present study, however, small crustaceans were found inside the bags with the collectors, which were possibly predators. Therefore, it is recommended that a new study be carried out, with the objective of studying the presence of predators in the bags with the collectors for *Nodipecten nodosus*.

According to TOWNSEND *et al*. (1991), after a certain period of time in the sea, the bivalve spats liberate their bissus and accumulate at the bottom of the bag, which can cause their death. This probably occurs due to the "bite" phenomenon, where the valves of an animal close down together with those of another animal damaging the soft areas of the spats. Besides this, the survival of the spats that are at the bottom of the bag can be affected by possible suffocation. In this way, it is recommended that studies are carried out to determine the moment in which *Nodipecten nodosus* liberates its bissus; this might help producers to avoid the damage described above.

CLAEREBOUDT *et al*. (1994) mention that the presence of fouling reduces the water flow inside of the bags which decreases the entrance of food. The organisms that generally compose the fouling serve as filters, which generates competition for food between the spats and the fouling. In the same way, its presence can reduce the supply of oxygen (WALLACE and REISNES 1985). Therefore, studies with the presence of fouling in the initial phase for the *Nodipecten nodosus* are recommended.

With the joined effect analysis, it was possible to observe that for spats with 15 days of settlement in laboratory, a great mortality rate occurred in the sea along 30 days. This fact was not observed for spats with 25 and 35 days of settlement in the laboratory, for which the mortality in the sea did not very throughout 30 days. URIARTE *et al.* (1996) also observed a higher mortality with time for younger *Argopecten purpuratus* spats, with average size smaller than 1 mm. According to BOURNE *et al*. (1989), during the settlement phase a high mortality rate occurs, and after this period as the animals grow there's a decrease in mortality.

Another important point that needs to be considered is the comparison between the two experiments which, in spite of some variations in methodology and in environmental factors, presented the same pattern of results.

For example, the recuperation average of spats thoughout the time in the laboratory was similar for both experiments 1 and 2, something which is expected in light of the fact that in the laboratory the environmental conditions are controlled.

For recuperation of spats in the sea, the same pattern of results was observed along the time periods, however, it is important to highlight that the data for recuperation averages of spats in the sea obtained in experiment 2 (Table 2) are higher than those of experiment 1 (Table 3). This can be attributed to various factors, such as the fact that the collectors used in experiment 1 were new and those of experiment 2 had already been used before, something which probably interfered in the settling because of the presence of biofilm in the collectors used.

This aspect can be supported by many results from different authors (FOIGHIL *et al*., 1990, PARSONS *et al.*, 1993, HARVEY *et al*., 1995, PEARCE

and BOURGET 1996, AVENDAÑO HERRERA *et al.*, 2002, AVENDAÑO HERRERA *et al*., 2003), who states that the biofilm has an important hole in the settlement induction and metamorphosis of scallop larvae.

Also, these values can be attributed to the food supplied in the settling phase, as well as to physical-chemical factors of the sea water, such as sea water temperature which presented average values of 18.5^{0}C in experiment 1 and 25.1^{0}C in experiment 2.

As can be seen in RUPP and PARSONS (2004) and RUPP *et al.* (2005), the total seston presented low values with 25% organic material and the chlorophyll **a** values indicates an energetically rich place to keep scallops spats. Thus, this study supports the conclusion that spats with between 15 and 25 days of settlement in laboratory present better results from the biological and economic view point.

After 15 days of settlement there is a higher recuperation of spats in laboratory, and for 25 days of settlement there is no variance along the time of recuperation of the animals in the sea. Regarding expenses, the best scenario is to keep the animals the shortest time possible in the laboratory. Despite the advantages of a laboratory with controlled environmental conditions for production, it demands the cultivation of microalgae in great quantities, as well as good asepsis of the local environment, which turns the process onerous and demands intensive labor.

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