Plate reduction in freshwater threespine sticklebacks (*Gasterosteus aculeatus*) - an adaptation to a different buoyancy regime?

Master of Science in Marine Biology

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ABSTRACT

One of the striking differentiations freshwater threespine sticklebacks (Gasterosteus aculeatus) with a marine origin experiences, is a reduction in the numbers of lateral plates. This has been demonstrated to be caused by factors such as differences in predation pressure, the access of important ions that build these lateral plates or by pleiotropic effects, such as osmoregulation. However, another factor could be important for stickleback moving from marine to fresh water, a difference in buoyancy and this has to be compensated for. In theory compensation for this alternation in buoyancy could be done in three different ways, through hydrodynamic lift, a change in swim bladder volume or by modifying the tissue density. To obtain information on how freshwater threespine sticklebacks adapt to this difference in buoyancy, a comparison of a marine stickleback population and two different freshwater stickleback populations, one completely plated and on low plated, were performed. Buoyancy, tissue density, swim bladder volume and mass of lateral plates were registered. All three populations of stickleback showed buoyancy near to neutral to their natural environment (marine or fresh water). This indicates that freshwater sticklebacks use other strategies than hydrodynamic lift to compensate for the reduced buoyancy. Further, comparing the swim bladder volume of freshwater low plated sticklebacks with marine completely plated sticklebacks demonstrated that they are of equally size. The tissue density of the freshwater low plated sticklebacks was lower than in the completely plated sticklebacks. These findings may demonstrate that the main strategy for sticklebacks with a marine origin in freshwater, is to reduce the tissue density rather than increasing the swim bladder volume. Mass measurements of lateral plates, which explains most of the differences in tissue density between freshwater low plated and marine completely plated sticklebacks supports this even further.

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1. INTRODUCTION

The threespine stickleback (*Gasterosteus aculeatus*) is a relatively small fish in the family *Gasterosteidae* (Sticklebacks) with the length of 11 cm as the longest recorded (Muus and Nielsen, 1999). The species has a wide distribution in both marine – and freshwater (lakes and streams) in the northern hemisphere. The marine populations found in the Baltic Sea, the Pacific and Atlantic Ocean, all are morphological similar with a well developed body armour (Bell and Foster, 1994). The body of marine populations has a complete row of 32 – 36 lateral plates on each side and strong spines both on dorsal and ventral side (usually 3 spines dorsally and 2 spines ventrally - Fig. 1A). The freshwater populations of stickleback are much more diverse. There are a few scattered populations and areas with sticklebacks resembling the marine ones, with completely plated body armour (Hagen and Gilbertson, 1972; Bell and Foster, 1994). However, far more common for the freshwater stickleback, is a reduction in both size and numbers of lateral plates. Freshwater populations are often isolated from marine sticklebacks and after a few generations a reduction of lateral plates and body armour can be seen (Bell et al. 2004). These new populations lack most of their lateral plates and body armour, and have only as few as 3 – 6 lateral plates on each side of the body (Fig. 1B). Some populations even show the ability to reduce all lateral plates (Münzing, 1963; Hagen and Gilbertson, 1972; Bell and Foster, 1994; Klepaker, 1995). The body colour of the threespine stickleback can often be cryptic and therefore varies with the habitat of the fish. The ventral side of the male becomes red during the breeding season, and the eye and body side have a bluish shine (Wootton, 1976; Reimchen, 1989).

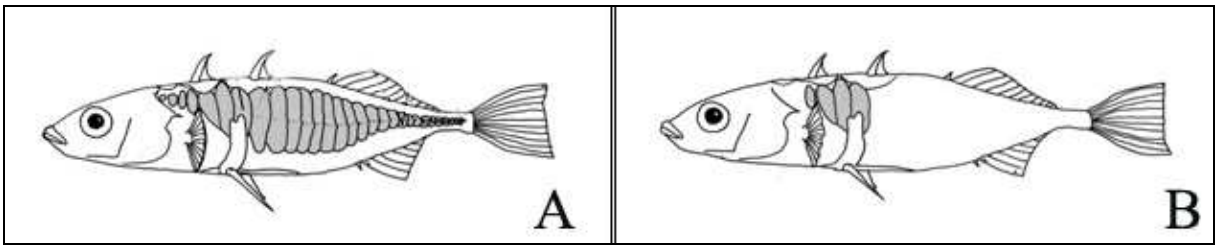

FIG. 1A. Marine and freshwater completely plated stickleback; **B.** Freshwater low plated stickleback

The phenomenon of a reduction of lateral plates and body armour in threespine stickleback has been given a great deal of attention during the last two centuries. William Yarrell describes five different species of sticklebacks already as far back as in 1836. These five species are today thought to be five different variations of the same species, the threespine stickleback. This first study has been followed up by newer studies on the subject by a vast variety of researchers (Bertin, 1925; Wootton, 1976; Bell and Foster, 1994; Östlund-Nilsson et al. 2007). The reduction in lateral plates has been demonstrated to be a result of a parallel evolution where freshwater populations are founded by marine sticklebacks isolated in freshwater (Colosimo et al. 2005). This reduction can be fast, with a significant loss of plates over just a few generations (Bell et al. 2004). The parallel evolution and the change in lateral plates and body armour can be explained genetically. Recessive genes in the marine stickleback populations codes for a reduction in lateral plates and body armour (Peichel et al. 2001; Colosimo et al. 2004). These genes are more favourable in fresh water and increase in frequency when the sticklebacks are isolated in the freshwater environment. What factors involved in this new freshwater habitat favours a reduction in lateral plate size and numbers?

Factors that could have an effect on this reduction must meet at least two basic demands. First, it must be factors that are common for a great deal of diverse freshwater habitats dispersed through out the sticklebacks' distribution area. Secondly, is must also be factors that differ significantly between the two habitats, marine – and fresh water. Previous studies have suggested that predators, predation pressure and predation defence could be such factors (Hagen and Gilbertson, 1972; Moodie and Reimchen, 1976). The access of calcium and other important ions for building lateral plates (Giles, 1983; Francis et al. 1986; Bell et al. 19939 and osmoregualtion and salinity tolerance are also studied as factors for this reduction (Heuts, 1947; Marchinko and Schluter 2007). However a factor that is rarely paid any attention in these kinds of studies, and that clearly meets both demands stated above, is the factor of buoyancy. Buoyancy is a factor that involves the density of the object and water in question. If the objects density is less than the density of the water, the object will have positive buoyancy. Opposite, if the density of the object is larger than the water, the object will have negative buoyancy. If the two are equal in density, the object will be neutrally buoyant. The density of the water varies mainly with salinity and temperature. Marine water with a salinity of 32 ppt and a temperature of 10 °C has a density of 1025 $kg*m^{-3}$, while freshwater (with a salinity of 0 ppt) at the same temperature has a density of 1000 $kg*m^{-3}$. This means that an

object with positive or neutral buoyancy in a marine environment well could be negative buoyant in freshwater.

An important way of regulating the buoyancy for fish is the evolution of a gas filled swim bladder. This swim bladder can be an open system, connected through a duct in the oesophagus, in physostome fish. These fish “fills” the swim bladder with gas from the water surface and expels it out direct into the water. An alternative system to this is a closed system, in physoclist fish. In this system the swim bladder is regulated by a gas gland (rete mirabile – gas in) and ovalen (gas out). The gas is secreted from the blood through the gas gland, and into the swim bladder, filling it with gas. To expel gas from the swim bladder again, ovalen is activated, and gas is secreted back into the blood vessels. Threespine stickleback belongs to the group of physoclist fish (von Ledebur, 1928; cited by Tait, 1960).

Marine fish that enter freshwater have three strategies to adapt their buoyancy to the new environment (Gee and Holst, 1992). First, they can adapt by using hydrodynamic lift, such as fin movements and swimming. Second, an increase of swim bladder volume will give a lower density and thereby a more positive buoyancy. The third strategy of adaptation is reducing body parts with a high density relative to the water, resulting in a reduction of tissue density. The first two strategies for adaptation are often seen in fishes entering from marine to freshwater during their lifespan (e.g. anadromeus fish). These strategies are observed in the two species *Culea inconstans* and *Pungitius pungitius*, where buoyancy regulation is a response to different salinities (Gee and Holst, 1992).

When marine fish is isolated in freshwater permanently, which has happened to threespine sticklebacks in numerous occasions, the fish have to adapt to their new environment on a permanent basis and the third strategy of buoyancy compensation is a possibility. This strategy is to reduce the body density by reducing the amount of structures which have a high density relative to water, like body structures (Webb, 1990). This leads to the research questions in this study. What adaptations do freshwater sticklebacks adopt in response of a new buoyancy regime? And can a loss of lateral plates in freshwater populations be a part of this adaptation? To address this, this study examines the density and buoyancy properties of both freshwater completely plated (without a reduction of lateral plates and body armour) and low plated (with a reduction of lateral plates and body armour) sticklebacks and relate these to density and buoyancy properties in marine completely plated sticklebacks. In addition, mass

registrations of the lateral plates are made to examine if these contribute in a significant way to the fish density and buoyancy.

2. MATERIALS & METHODS

2.1 THREESPINE STICKLEBACK SAMPLING

Threespine sticklebacks (*Gasterosteus aculeatus*) were collected from three different localities, thereby sampling three different populations (overview, Fig. 2). The marine completely plated sticklebacks were collected in Førdespollen, Sotra (Fig. 3) on the 24th and 25th of April 2008, the completely plated freshwater sticklebacks collected in Lake Myrdalsvatnet, Bergen (Fig. 4) on the 6th and 7th of May 2008, and the freshwater low plated sticklebacks were collected in Lake Liavatnet, Bergen (Fig. 5) on the 5th and 7th of May 2008. Sixty sticklebacks of each population were collected, and from these sixty, twenty sticklebacks of each population were used in laboratory work.

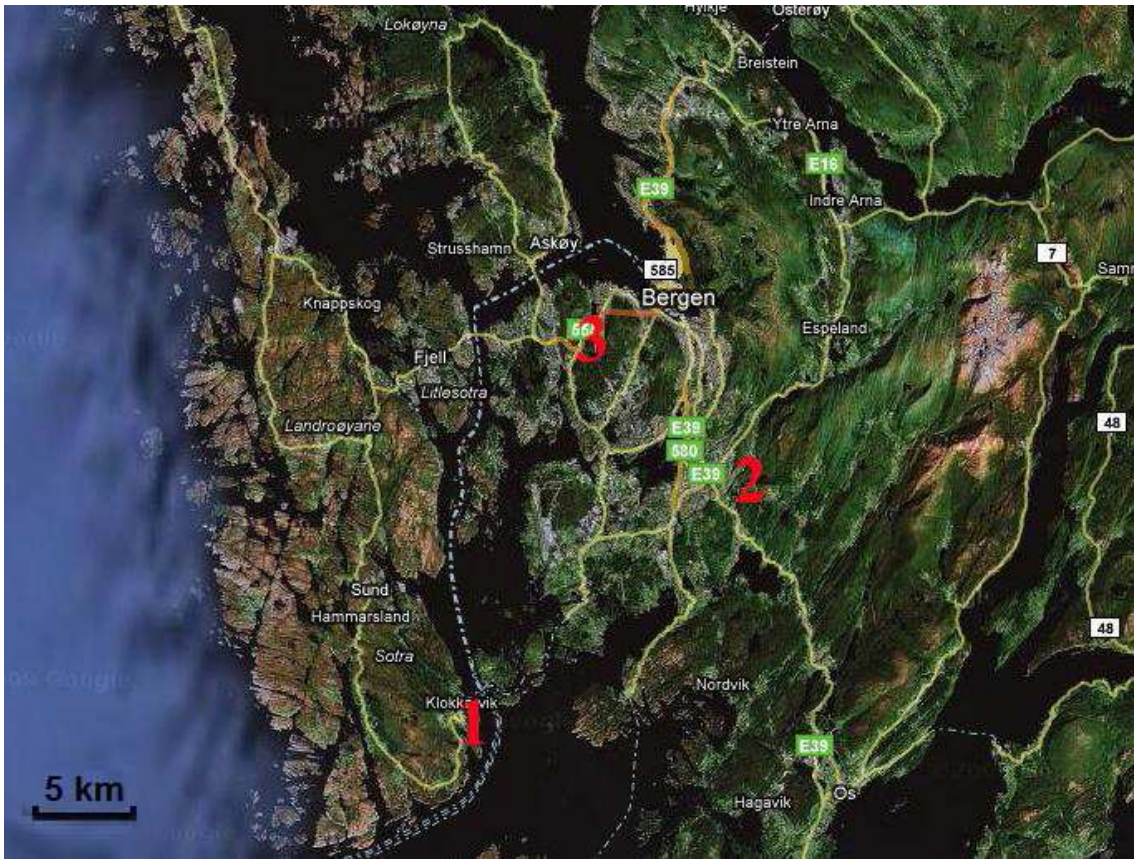

FIG 2. An overview over sampling areas for threespine stickleback. 1. Førdespollen, marine completely plated stickleback; 2. Myrdalsvatnet, freshwater completely plated stickleback; 3. Liavatnet, freshwater low plated stickleback

FIG. 3. Førdespollen, red circle marks the sampling site for marine completely plated sticklebacks

FIG. 4. Myrdalsvatnet, red circle marks the sampling site for freshwater completely plated sticklebacks.

FIG. 5. Liavatnet, red circle marks the sampling site for freshwater low plated sticklebacks.

2.2 SAMPLING METHODS

A plastic fry trap (Breder, 1960) was used to sample the freshwater populations of the threespine sticklebacks. The size on these traps was 50 x 50 x 100 cm and consists of a clear plastic box with two longer winglike structures which continue inward to form a re-entrant split (Fig. 6A). The traps were placed on the sampling sites during the early hours of the first sampling day on each locality and collected the following day. The plastic fry traps works by guiding the fish towards and trough the re-entrant split, thereby making it almost impossible for them to escape back out. The bottom of these traps is constructed in a way that allows the traps to be drained without a loss of sampling catch. When sampling the marine completely plated sticklebacks, a large landing net (100 cm in diameter) was also used, in addition to the plastic fry traps. Sticklebacks with the size between 30 and 60mm, and without visible endo- and ectoparasites, were collected and put in a plastic container (Fig. 6B) ready for transportation to Bergen High Technology Centre (BHTC).

FIG. 6A. Sampling marine completely plated sticklebacks by using plastic fry traps; **B.** Transportation container for threespine sticklebacks

2.3 LABORATORY WORK

The sticklebacks were brought back to BHTC after sampling, where the marine sticklebacks were kept in a flow-through system shown by Fig. 7, and the freshwater sticklebacks were kept in 60 litres aquariums with air supply until the were to be used in the laboratory. The flow-through system allows a continued change of water, coming in from the sea outside the facility. $^{1}/_{3}$ of the water in the freshwater aquariums was changed once a week. The water temperatures in these systems/aquariums were 12 °C (± 1 °C). Both the marine- and the freshwater sticklebacks received a daily amount of red mosquito larvae, and starved for one day a week.

FIG. 7. The flow-through system where the marine threespine sticklebacks were held

The sticklebacks were starved for a period of 24 hours before buoyancy and density registrations were made to make sure that the sticklebacks didn't have food left in their digestion system, this because any leftover food in the intestine could influence the mass of the fish. After this period the fish was euthanized by using a lethal dose of MS-222 (ethyl m-amino-benzoate, 40 mg pr. 100 ml H_20). The sticklebacks used for experiments were picked randomly, and had a size variation from 36mm to 54mm.

2.3.1 BUOYANCY MEASUREMENTS

The sticklebacks were weighed by using Sartorius Genius Series ME5 with YDK 01 setup (Fig. 8A & B), finding the mass of the fish with the swim bladder still intact (ISW) in non-ionic water. The fish were dried with a paper towel and photographed by using a Nikon D70s with a 90mm Tamron macro lens. Then the sticklebacks mass in dry condition, were found by using a Sartorius BP61S. The fishes mass in non-ionic water with a punctured swim bladder (PSW) were found by puncturing the swim bladder by using a syringe, and have the swim bladder filled with water.

FIG 8A. Sartorius Genius Series ME5 with YDK 01; **B.** YDK 01 setup while measuring a stickleback

2.3.2 DISSECTING LATERAL PLATES AND PELVIS

A dissection of the sticklebacks' lateral plates (Fig. 9) from plate 8 to 34 and ventral spines were performed. This done out by using a small scalpel, twisters and a Wild Heerbrugg binocular with an Intralux 6000 as a source of light. Lateral plates were dissected on both side of the first 3 fishes, and tested for differences between the mass of plates on each side. This showed little difference between the two sides, so only one side was registered for the remaining specimens (and then multiplied by two). Each lateral plate was carefully removed under the binocular by hand, and scraped free of any leftover tissue. At the posterior end (the keel) the plates are small and difficult to dissect, so a dissection of the plates as a group was performed, scraping them free of tissue. In the results posterior plates from plate 21 and back are defined as the keel. The pelvis with pelvic spines was cut carefully just above the connection between plate nr 7 and ascending branch of the pelvis. The mass of each of the lateral plates and pelvis was found by using a Sartorius micro M3P, shown by Fig. 10. After these measurements the plates and spines were put into small jars and registered for storing. The total mass of lateral plates pr. fish could now be calculated, and thereby also the relationships between amounts of lateral plates versus swim bladder volume.

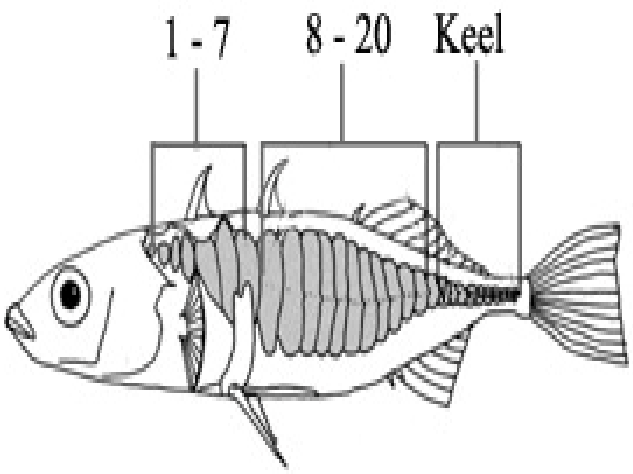

FIG. 9. Lateral plates and ventral spines in a completely plated threespine stickleback

FIG. 10. Satorius micro M3P

2.4 TREATMENT OF DATA

2.4.1 DENSITY AND BUOYANCY CALCULATIONS

The density was calculated using the formula:

$$p = \frac{m(a) \cdot [p(fl) - p(a)]}{m(a) - m(fl)} + p(a)$$

p: fish density
p (fl): density of water for a given temperature
p (a): density of air (0.0012 $^{g}/_{cm^3}$ at 20.0 °C)
m (a): body mass of fish in air
m (fl): mass of fish in water

The swim bladder volume could now be found by:

$$V(sb) = \frac{m(psw) - m(isw)}{p(fl)}$$

V (sb): volume of swim bladder
m (psw): mass of fish in water with punctured swim bladder
m (isw): mass of fish in water with intact swim bladder
p (fl): density of water for a given temperature

To achieve neutral buoyancy in water the body mass of the fish has to be equal to the volume of water that it displaces. The swim bladder volume of a neutral buoyant fish can be calculated by using the formula (Strand et al. 2005):

$$V(n) = \frac{m(a) \cdot (1 - p(fl)/p)}{p(fl)}$$

V(n): swim bladder volume of neutral buoyant fish

Relative buoyancy could now be calculated as a buoyancy force by the formula:

$$B = \left[V(sb) - V(n)\right] \cdot p(fl) \cdot g$$

g: earth gravity (9.81 ms^2)

The results from the mass registrations of lateral plates and pelvis were put into worksheets in Microsoft Excel, and the program was used to calculate the mean- and standard deviation values.

2.5 STATISTICS

A two-tailed t-test in SPSS 16.1 was used to determine the significance in tissue density between the marine full plate, freshwater full plate and low plate sticklebacks. The two-tailed t-test was also used to determine if there was a significant difference in swim bladder volume and buoyancy. A one sample t-test with the same program (SPSS 16.1) was also carried out to see if there was a significant difference in the three different variations of fish density and the water density. A t-test was also performed to see if there were significant differences between the swim bladder volumes of the different populations of fish, and the differences between the lateral plates of the variations were also tested.

3. RESULTS

3.1 GENERAL DATA

Data was obtained from twenty sticklebacks of each group. One fish in the marine stickleback group had to be excluded from further analysis due to methodical error (mass measurements for fish nr. 20 with punctured swim bladder was not obtained), so only nineteen marine sticklebacks were included in the analysis (Table 1 and 2 - for all raw data see Appendix I). The marine sticklebacks are larger than the freshwater sticklebacks, both measured in body mass and total body length. The completely plated freshwater population has the highest variation in size.

The marine sticklebacks have a larger pelvis mass than the freshwater completely plated sticklebacks, but adjusted for the size difference, the pelvis of the two groups of completely plated sticklebacks are equal in size (2,1 percent of body mass). The low plated sticklebacks have a smaller pelvis than the completely plated, for these the pelvis is 0,6 percent of the body.

TABLE 1. An overview of mean values of the raw data of wet mass and dry mass with intact swim bladder (ISW) of the different populations of threespine sticklebacks, mean length, mean mass of ventral spines and fish volume

Fish	N	Mean mass, ISW wet	Mean mass, ISW dry	Mean mass, pelvis	Mean fish total length	Fish volume
Marine completely plated	19	26,309	957,621	19,983	48,6	0,899
Freshwater completely plated	20	-2,413	735,763	15,541	44,0	0,694
Freshwater low plated	20	-1,310	679,097	4,384	40,4	0,645

NOTE: All mass are in mg, length in mm, and volume in ml

TABLE 2. An overview of mean values for the mass of each specific lateral plate at each population of threespine stickleback

Fish	Mean plate 8	Mean plate 9	Mean plate 10	Mean plate 11	Mean plate 12	Mean plate 13	Mean plate 14
Marine completely plated	0,781	0,624	0,566	0,489	0,452	0,397	0,342
Freshwater completely plated	0,690	0,531	0,477	0,424	0,381	0,319	0,284
Fish	**Mean plate 15**	**Mean plate 16**	**Mean plate 17**	**Mean plate 18**	**Mean plate 19**	**Mean plate 20**	**Mean keel**
Marine completely plated	0,287	0,229	0,190	0,153	0,132	0,106	0,990
Freshwater completely plated	0,245	0,192	0,153	0,125	0,110	0,084	0,808

NOTE: All mass are in mg; the freshwater low plate population is removed because of a constant value of 0, due to lack of lateral plates from 8 - 20

3.2 FISH BUOYANCY

To see if there was a difference in buoyancy in the different groups of sticklebacks, the mean fish densities were compared to the density of the water in their natural environment. The results showed that the marine fish had a higher density (1024,681 g*cm-3) than both the freshwater variations (994,472 g/cm^3 for the freshwater completely plated and 995,275 g/cm^3 for the freshwater low plated), which were similar. The buoyancy for marine completely plated fish was 0,001 N. For freshwater completely plated fish the buoyancy was 0,005 N and for freshwater low plated 0,004 N. This shows that all populations of fish are slightly positive buoyant, but close to neutral (Table 3 & Fig. 11). The higher buoyancy in the both of the freshwater populations could be due to a methodical error (air bubbles in gill areas, on the skin surface, etc.). The density of salt/freshwater was taken into consideration when calculating the buoyancy (density marine environment: 1024.287 g/cm^3; freshwater environment: 999.526 g/cm^3).

TABLE 3. The different populations of threespine stickleback buoyancy in their natural environment

Fish	Mean density ISW	St.Dev Denisty	Buoyancy force	St.Dev Boyancy force
Marine completely plated	1023,681	0,005	0,001*	0,036
Freshwater completely plated	994,472	0,002	0,005*	0,003
Freshwater low plated	995,275	0,003	0,004*	0,003

NOTE: Swim bladder density in g/cm^3; * calculated from formula described in chapter 2 - where the density of freshwater is 999.526 g/cm^3 and ocean water 1024.287 g/cm^3; Buoyancy force in Newton (N)

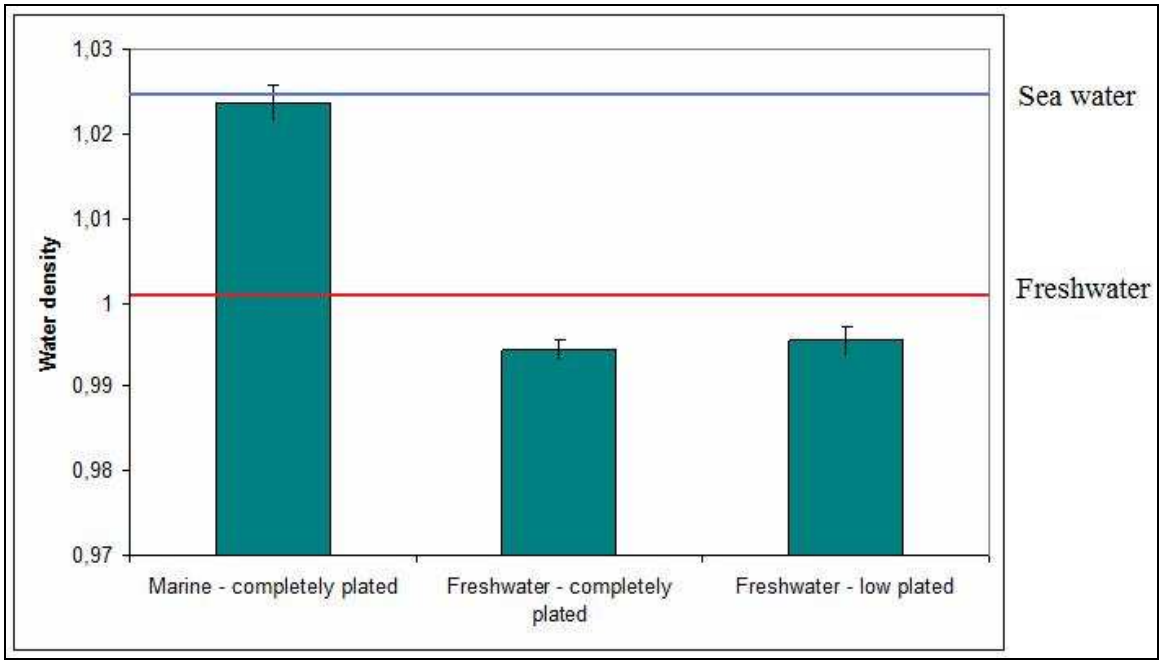

FIG 11. Mean fish density of marine completely plated, freshwater completely plated and freshwater low plated sticklebacks with standard error. The lines mark the density of marine- and fresh water

3.2.1 SWIM BLADDER VOLUME

To find out if there was a difference between the swim bladder volumes among the populations of fish, the method described in 2.4.1 Buoyancy measurements were used for calculations. The results from the calculations (Table 4 and Fig. 12) showed that mean volume of the swim bladders were similar for the marine (0,034 ml) and the freshwater low plated (0,035 ml) group of sticklebacks, but it is here not adjusted for size difference. The freshwater completely plated fish had a greater volume inside their swim bladder (0,054 ml).The percentage of the swim bladder compared to the total volume of fish was also calculated. The result was 4,2 for the marine completely plated, 7,9 % for the freshwater completely plated and 5,5% for the freshwater low plated population.

TABLE 4. Mean value, standard deviation and percentage of the swim bladder for the different populations of threespine stickleback

Fish	Mean volume swim bladder	St.Dev	Swim Bladder %
Marine completely plated	0,034	0,014	4,2
Freshwater completely plated	0,054	0,026	7,9
Freshwater low plated	0,035	0,014	5,5

NOTE: Swim bladder volume in ml

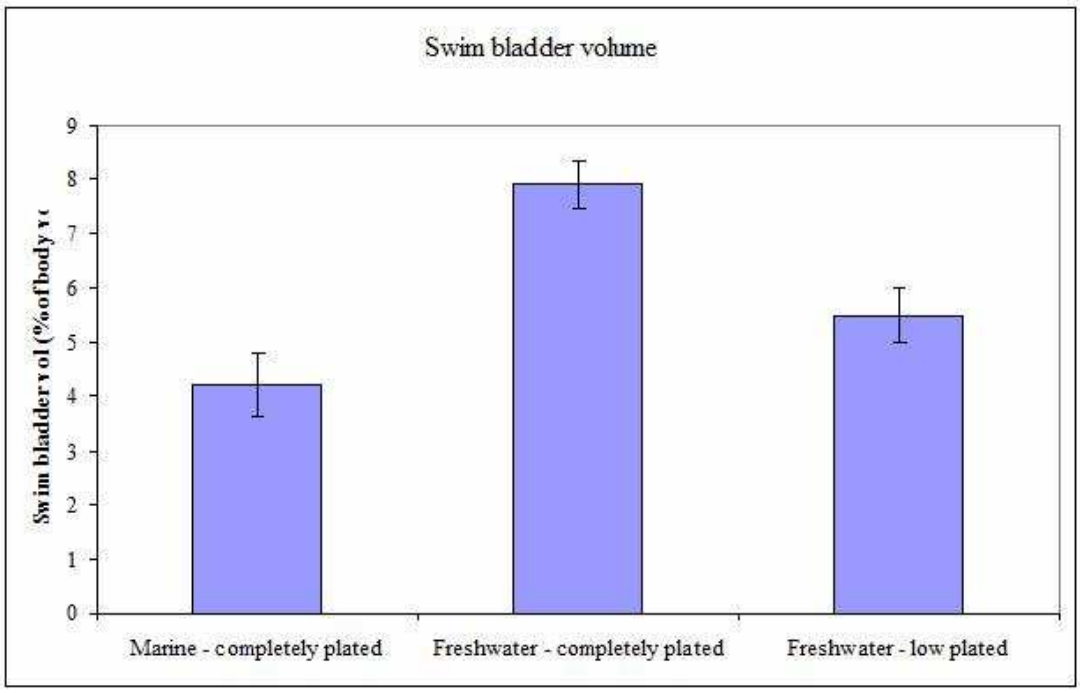

FIG. 12. Swim bladder volume of marine completely plated, freshwater completely plated and freshwater low plated sticklebacks

To test if the differences in swim bladder volume were significant or not a two-tailed t-test was performed between the different populations of threespine sticklebacks (Table 5). The test showed that there was a significant difference both between the marine completely plated fish and the freshwater completely plated (p-value < 0,001), and the freshwater completely plated fish and the freshwater low plated (p-value < 0,001). It is a smaller, but also significant difference between marine completely plated and freshwater low plated (p-value = 0,001).

Buoyancy differences were also tested with two-tailed t-test. The results were significantly different both between marine completely plated sticklebacks and freshwater completely plated (p-value < 0,001), and between marine completely plated sticklebacks and freshwater low plated (p-value = 0,006). Comparing buoyancy between the two freshwater populations (completely plated and low plated) gave no significant difference (p-value = 0,239).

Tissue density differences were tested with a two-tailed t-test to see if there was a significant difference between the three different populations of threespine sticklebacks. All three combinations of testing showed a significant difference. The p-value was 0,026 between marine completely plated fish and freshwater completely plated. Marine completely plated

and freshwater low plated showed a p-value of 0,010. The comparison of the two freshwater populations gave a p-value of 0,001.

TABLE 5. P-values for different two-tailed t-tests; marine completely plated vs. freshwater completely plated sticklebacks; marine completely plated vs. freshwater low plated sticklebacks; freshwater completely plated vs. freshwater low plated sticklebacks

T-test between:	Swim bladder volume	Buoyancy	Tissue density
Marine completely plated vs. Freshwater completely plated	<0,001*	0,001*	0,026*
Marine completely plated vs. Freshwater low plated	0,001*	0,005*	0,010*
Freshwater completely plated vs. Freshwater low plated	<0,001*	0,239	0,001*

NOTE: * A significant difference in p-values

3.2.2 FISH DENSITY (ISW) COMPARED TO DENSITY OF MEDIUM

The mean fish density (with intact swim bladder – ISW) in each population was compared to the density of the water the different populations of fish lived in. The results showed that the difference was small in all three groups of sticklebacks. The marine completely plated population had a difference of 0,001, the freshwater completely plated 0,005 and the freshwater low plated 0,004 (Table 6).

TABLE 6. The comparison of mean density of fish with intact swim bladder (ISW) and water density

Fish	Mean fish density ISW	Water density	Difference
Marine completely plated	1,024	1,024	0,001
Freshwater completely plated	0,994	1,000	0,005
Freshwater low plated	0,995	1,000	0,004

NOTE: density in g/cm^3

To check if there was a significant difference between the fish density of the populations and the density of the water the fish lived in, a one sample t-test using SPSS 16.1 was performed. This test showed that the marine completely plated sticklebacks are not significant different than their natural environment (p-value = 0,721). The two freshwater populations of sticklebacks however show a significant difference compared to their environment (p-value < 0.001).

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3.3 TISSUE DENSITY

3.3.1 PUNCTURED SWIM BLADDER (PSW)

The density for the different fish populations with their swim bladder punctured (tissue density) was also measured. Tissue density was highest in the freshwater completely plated sticklebacks (1,073 g/cm^3), then the marine completely plated sticklebacks had a tissue density of 1,066 g/cm^3, and the freshwater low plated sticklebacks had the lowest tissue density of 1,050 g/cm^3 (Fig. 13). The difference between the low plated sticklebacks and the two populations of completely plated sticklebacks was significant (two-tailed t-test, p-value < 0,001). The two populations of completely plated sticklebacks were not significantly different (two-tailed t-test, p-value = 0,09).

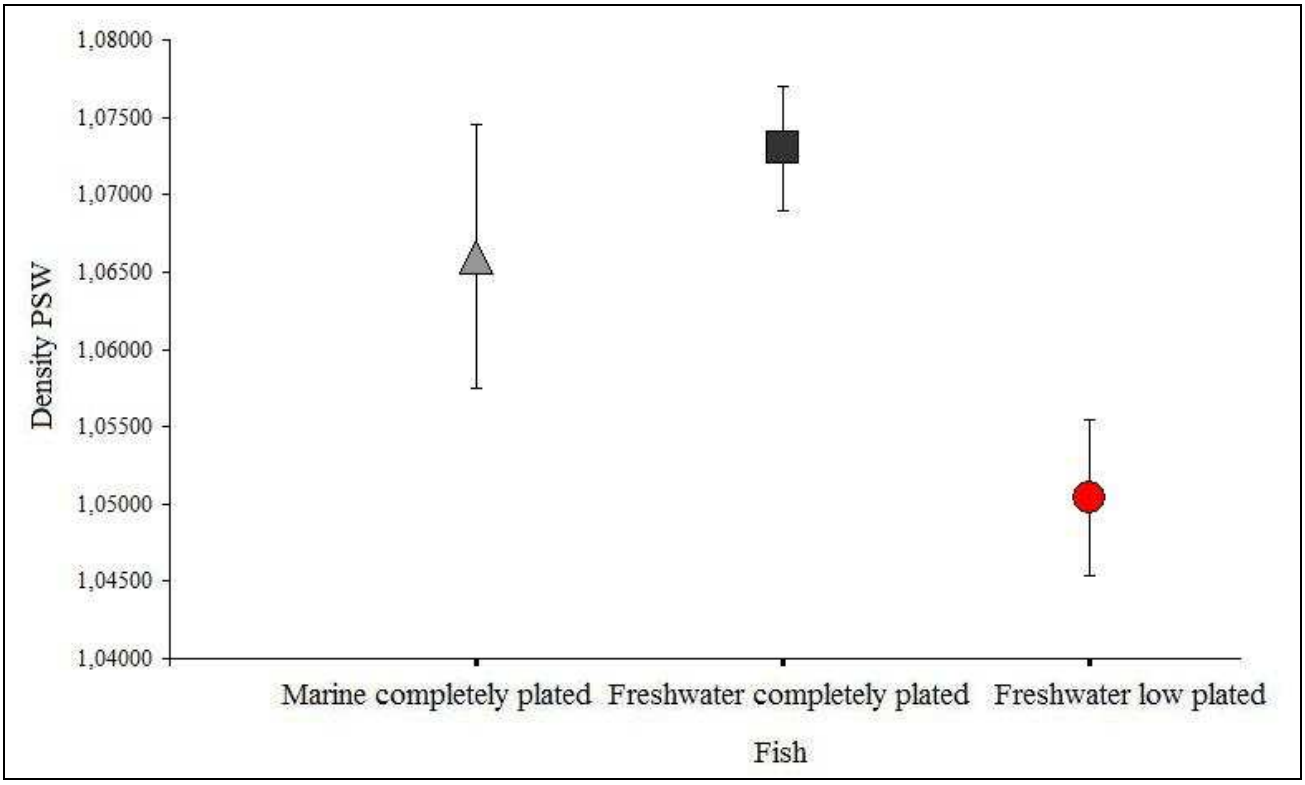

FIG. 13. The fish density with a punctured swim bladder (PSW) in the different populations of threespine stickleback, standard deviation also showing

3.3.2 MASS OF LATERAL PLATES

The mass of lateral plates was registered and the results showed, when size difference was taken into consideration, that the percentage of mass of lateral plates compared to body mass were close to equal (Table 7).

TABLE 7. Percentage of lateral plates of the total mass of the different populations of threespine stickleback

Fish	Mean mass of plates	St.Dev	Mean mass of fish	lateral plates / body mass (%)
Marine completely plated	11,474	0,523	956,5	1,2
Freshwater completely plated	9,643	0,444	746,7	1,3

NOTE: all mass are in mg; the freshwater low plate variation removed due to the fact that they lack lateral plates

3.3.3 A HYPOTHETICAL REDUCTION IN LATERAL PLATES AND PELVIC APPARATUS

Reducing the mass of lateral plates in the two completely plated populations of sticklebacks, thereby reducing the body mass of fish, would have an effect on the density of the sticklebacks. This reduction would hypothetically bring the density of the marine completely plated and freshwater completely plated sticklebacks closer to the low plated sticklebacks. A hypothetical reduction of lateral plates were performed by reducing the plates from plate eight and backwards, ending up reducing the keel. The results showed that the more lateral plates which were reduced in the two completely plated populations (marine and freshwater completely plated), the more similar to the freshwater low plated population they got (Table 8 and Fig. 14). At the end, when every plate was removed, the reduction showed that the marine completely plated population was close to similar to the freshwater low plated fish, at 1,053 $g*cm^{-3}$. Also the freshwater completely plated population got close (1,060 $g*cm^{-3}$).

TABLE 8. Mean hypothetical reduction in the lateral plates (density, $g*cm^{-3}$) in the different populations of threespine stickleback - The 20th plate is the first to be reduced, then the 19th plate and so on. The keel is the last to be reduced.

Fish variation	Plate 20	Plate 19	Plate 18	Plate 17	Plate 16	Plate 15	Plate 14	Plate 13	Plate 12	Plate 11	Plate 10	Plate 9	Plate 8	Keel -
Marine completely plated	1,067	1,066	1,066	1,065	1,065	1,064	1,064	1,063	1,062	1,060	1,059	1,057	1,056	1,053
Freshwater completely plated	1,073	1,073	1,072	1,072	1,071	1,071	1,070	1,069	1,068	1,067	1,066	1,064	1,062	1,060
Freshwater low plated	1,050	1,050	1,050	1,050	1,050	1,050	1,050	1,050	1,050	1,050	1,050	1,050	1,050	1,050

NOTE: All plates are in density (density, g/cm^3)

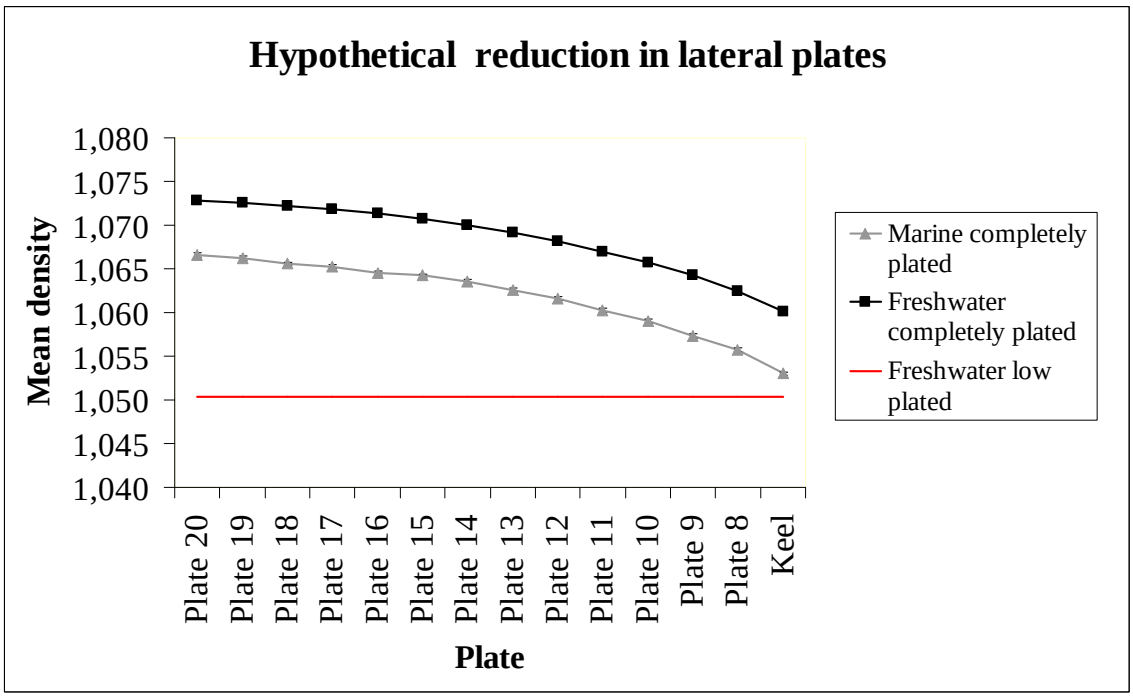

FIG. 14. Hypothetical reduction of the lateral plates in the threespine stickleback populations

If a reduction of the pelvis also was to be calculated for, and thereby bringing the densities of the completely plated populations down even further, the marine completely plated and freshwater completely plated sticklebacks would approach the low plated population even more.

4. DISCUSSION

There are many studies done on the threespine stickleback, and a number of these looks at the phenomenon of lateral plate reduction (Yarrell, 1836; Bertin, 1925; Wootton, 1976; Bell and Foster, 1994; Östlund-Nilsson et al. 2004). In freshwater sticklebacks, it is demonstrated that this lateral plate reduction is a result of a parallel evolution where populations of marine fish become isolated in freshwater and forms new populations (Colosimo et al. 2005). Studies also show that these stickleback populations, isolated in freshwater, during a few number of generations are capable of reducing their lateral plates and armour (Bell et al. 2004). This capability could be explained by some recessive genes expressing plate reduction being present in marine populations in low frequency (Peichel et al. 2001; Colosimo et al. 2004).

Previous studies done on the geographical distribution of the threespine stickleback show that species thrive in the ocean, lakes and streams all over the northern hemisphere (Bell and Foster, 1994). Some of these studies assume that freshwater populations with reduced lateral plates and body armour are linked to streams and lakes in warmer climate (Bell, 1982; Hagen and Moodie, 1982; Baumgartner and Bell, 1984; Hagen, 1987; Baumgartner, 1992), but newer studies also show that these populations dominate in colder climate, as Norway and Alaska (Klepaker, 1995; Bell et al. 2004). When looking at previous studies done on predation and predation pressure, also thought to be a factor for lateral plate reduction, these show that whenever freshwater low plated populations have a high predation pressure, the fish adapts to this by having a higher number of lateral plates than other low plated populations in lakes nearby without heavy predation pressure (Hagen and Gilbertson, 1972; Moodie and Reimchen, 1976). The access of important ions such as calcium is also thought to be possible factor impacting the ability to develop lateral plates and body armour. Studies have shown that in some populations where there is a low concentration of calcium (Ca super (2+) - less than or equal to 2 multiplied by 5 mg/l), the populations have a significant reduction of lateral plates (Giles, 1983). Also the salinity concentration has been investigated as a possible factor for the lateral plate reduction. Heuts (1947) worked out a hypothesis that low salinity in freshwater forces the sticklebacks in these habitats to reduce bony structures such as lateral plates and pelvic and dorsal spines. A later study shows that salinity influences juvenile growth in freshwater (Marchinko and Schluter 2007). The population of sticklebacks with reduced lateral plates grew as much as 65 % faster than the completely plated population. All

of the factors above can be of importance for the reduction of lateral plates and body armour in the freshwater threespine stickleback, but why this reduction occurs in so many different habitats of freshwater (with different predation pressure, calcium levels, salinity, etc.) is unclear. It is therefore maybe a need to look at other factors as well.

Searching the literature with the topic of buoyancy and buoyancy regulation in this group of fish doesn't return with many answers. Two articles were found showing the sticklebacks capability of regulating their own buoyancy. An article from Beaver and Gee (1988) described that a change in swim bladder volume as a response to a difference in water current where shown in the two species *Culaea inconstans* and *Pungitius pungitius*. These two species, *C. inconstans and P. pungitius*, also have regulation of swim bladder volume, after a longer period of acclimatizing, to changing water densities and salinities (Gee and Holst, 1992). When looking at the relevance of lateral plates and swim bladder volume in sticklebacks, the only reference found was an article by Mori (1987) where he points out a question if the lateral plates are heavy for the sticklebacks in freshwater, without following this through.

The swimming pattern of a threespine stickleback is a short period of swimming followed by a longer period of hovering without much fin movement. Not surprisingly does this study give a good indication that three spine sticklebacks do not use hydrodynamic lift to compensate for the reduced density, and thereby reduced buoyancy, when living in freshwater. The results show that the freshwater threespine sticklebacks are slightly positive buoyant in water, something that are somewhat puzzling. These results may include small margins of error, possibly due to tiny air bobbles left in the swim bladder after puncturing it, when measuring the mass of the fish with density close to water density in distilled water. Most likely will both marine- and freshwater sticklebacks respond in the same manner to different water densities and become close to neutral.

This study shows that hydrostatic mechanisms are used to achieve neutral buoyancy in both the freshwater completely plated and low plated sticklebacks, however in different ways. The completely plated fishes increases the volume of their swim bladder compared to the marine sticklebacks, as a solution to the buoyancy issue, and thereby increasing their uplifting force. The low plated fishes however use a reduction of tissue density, something that results in a reduced down pulling force. Could this tissue density reduction in freshwater low plated

sticklebacks happened because of their lack of lateral plates? Or is this reduction of lateral plates insignificant to the tissue density, simply because the mass of lateral plates are too small? This study clearly shows that the mass of lateral plates highly affects the density of the fish, and relative to the marine completely plated sticklebacks it alone can account for $^{2}/_{3}$ in the difference in body density. It is not only the number of lateral plates that are reduced in the freshwater low plated sticklebacks, but also the size of these and the size of pelvis and spines (Bell and Foster, 1994). The results show that the freshwater low plated sticklebacks compensate the reduced buoyancy in freshwater contra a marine environment by reducing their heavy armour, and thereby making them selves less dense.

This strategy shown by the freshwater low plated population of sticklebacks, a reduction of both number and size in lateral plates, size of spines and thereby a total reduction of body armour must have a selective advantage compared to the alternative solutions to the problem encountered by the fish, such as increasing the swim bladder volume. The strategy of reducing body armour must also be large enough to compensate for this taken the increased predator risk into consideration. An alternative strategy will be an increase of the swim bladder volume. As sticklebacks are physioclists, it will cost energy to fill the swim bladder from gases dissolved in the blood, and an increased swim bladder volume will cost more energy to fill. That being said maintaining the gas inside the increased swim bladder is relatively cheap and do not affect the energy usage (Harden Jones and Scholes, 1985). But a larger swim bladder volume will affect the space left in the confined abdominal cavity, and the fish may be faced with some trade-offs. One will be the trade-off between larger swim bladder volume and a lower stomach capacity. A study on Atlantic cod (*Gadus morhua*) showed that the stomach content influenced the swim bladder volume capacity, and thereby the cod's ability to control its buoyancy (Ona, 1990). The fuller the stomach got in these cods, the less gas was able to maintain inside the swim bladder. A second trade-off especially the female fish will have to take into consideration will be the trade-off between swim bladder volume and the volume of eggs. A larger swim bladder will allow less space for eggs in the abdominal cavity, and thereby reducing the fish's fecundity.

Another problem the sticklebacks will have to deal with if they chose the strategy of increasing their swim bladder volume will be altered stability point. With a larger swim bladder the fish will have a buoyancy centre below the centre mass of the fish, and thereby be more instable and have a greater chance to roll (Goldberg, 1988; Eidietis et al. 2002). This roll

has to be corrected for by fin movements, which again cost energy for the fish. Without this correction by the fish, it will roll over on its side as observed when sedated. Studies done on the tendency to roll shows that the bigger the distance between the two metacentric height centres get, the greater the chance of roll, this may be one problem the freshwater completely plated sticklebacks (from Lake Myrdalsvatn) is facing with their strategy, of an increase of swim bladder volume, for staying buoyant in the less dense freshwater. Because of this increase the centre of buoyancy will move further down in the fish, thereby altering the metacentric height (making it higher), and ultimately make the fish more unstable. If this alternation is of any significantly cost for the sticklebacks is unknown, as is the freshwater completely plated sticklebacks answer to the issue at hand, and both of these topics has to be looked at closer in future studies.

So how could the occurrence of freshwater completely plated sticklebacks be explained if the strategy of a reduction of body armour is so successful? The answer may be location. The type of climate which the sticklebacks lived in was earlier thought to be the reason for low plate populations. Both studies from Hagen and Moodie (1982) and Baumgartner and Bell (1984) shows that low plated freshwater sticklebacks thrive in warmer climate. The reduction of lateral plates and body armour were therefore thought to be more important for sticklebacks living in areas with warmer water, maybe due to the difference in density between warm and cold water. Streams and lakes with a current are also places where low plated sticklebacks are living (Bell, 1982; Hagen, 1987; Baumgartner, 1992), and with the sticklebacks *Culaea inconstans* and *Pungitius pungitius* it is documented that they reduce their swim bladder volume with as much as 80 % (Beaver and Gee, 1988). Newer literature have made this climate assumption less clear, and shows that these kinds of populations also occur in northern parts of the world, without strong water current. This is places such as Norway and Alaska (Klepaker, 1995; Bell et al. 2004). In still water the advantage of a low plated body may not be as significant as in streams, and that may be a reason why there are completely plated sticklebacks in Lake Myrdalsvatn. The answer could be quite simple. The population here, given the fact that the lake is well above the maximum postglacial sea level (Lohne, 2005), is probably formed by some specimens of completely plated sticklebacks released into the lake. This may have resulted in a small gene pool, with a lack of the genes for reduction of lateral plates and armour, which again have resulted in a population of completely plated fish. Then as a possible adaptation to the less dense freshwater the sticklebacks have increased their swim bladder volume and kept the body armour.

It is difficult to say wither or not lateral plates and body armour reduction is a primary response or just one of many side effects to the regulation of buoyancy for the fish. Other factors, yet unknown, may also be important for the reduction. Anyway it's still a fact that buoyancy and buoyancy control is a really important issue aquatic organisms/animals have to deal with, and the same species may have different solutions to this issue in different habitats. It is already shown by Eastman and Deveries (1982) that fish are able to reduce their body structures in order to maintain buoyancy. This taken into consideration together with the results given in my study it looks like buoyancy regulation is a possible and plausible mechanism contributing to armour reduction in freshwater three spine sticklebacks. Of curse this will need further studies in the future.

5. CONCLUSION

Many factors influence the lateral plates and body armour reduction in threespine sticklebacks with a marine origin, when they move into fresh water. In which degree of importance the different factors contribute, and what the primary response for this reduction is, is not clear. The answer could be different for different habitats and areas. This study demonstrates that the freshwater threespine sticklebacks can use hydrostatic methods as a strategy for maintaining close to neutral buoyancy. It is also demonstrated that a reduction of lateral plates and body armour can be a way of adapting to water of less density. If this is a primary response or just one of many important factors involved in the process is difficult to say. Still, buoyancy regulation seems to be a possible and plausible mechanism contributing to lateral plates and body armour reduction in freshwater threespine sticklebacks.

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Formula for calculating water density: http://www.csgnetwork.com/h2odenscalc.html

Picture on front page: Photo by Johnny Jensen

Fig. 2, Fig. 3, Fig. 4, Fig. 5: www.maps.google.no

APPENDIX I

TABLES FOR RAW-DATA OF THREESPINE STICKLEBACKS

TABLE 9. Raw-data for the threespine sticklebacks in this study. Morph 1.1 – 1.20 are marine completely plated sticklebacks (Morph 1.9 was not included in the calculations due to measuring error), morph 2.1 – 2.20 are freshwater completely plated sticklebacks and morph 3.1 – 3.20 are freshwater low plated sticklebacks

Fish	Mass, wet(g)	Mass, w II(g)	Mass, w III(g)	Mass, dry(g)	Mass, d II(g)	Mass, d III(g)
Morph 1.1	0,0228	0,0225	0,0220	0,9674	0,963	0,9616
Morph 1.2	0,0247	0,0250	0,0238	1,2701	1,267	1,2644
Morph 1.3	0,0200	0,0181	0,0184	0,7245	0,722	0,7206
Morph 1.4	0,0233	0,0256	0,0256	0,8976	0,896	0,8940
Morph 1.5	0,0273	0,0253	0,0258	1,1508	1,149	1,1475
Morph 1.6	0,0186	0,0209	0,0215	0,8218	0,822	0,8205
Morph 1.7	0,0182	0,0178	0,0163	0,7068	0,706	0,7053
Morph 1.8	0,0178	0,0178	0,0179	0,6455	0,645	0,6446
Morph 1.9	x	x	x	x	x	x
Morph 1.10	0,0247	0,0243	0,0243	0,9475	0,947	0,9454
Morph 1.11	0,0280	0,0270	0,0269	1,0859	1,085	1,0843
Morph 1.12	0,0294	0,0286	0,0299	0,7884	0,787	0,7859
Morph 1.13	0,0289	0,0303	0,0286	1,1785	1,178	1,1766
Morph 1.14	0,1480	0,0149	0,0155	0,7465	0,746	0,7449
Morph 1.15	0,0303	0,0304	0,0312	1,2267	1,226	1,2247
Morph 1.16	0,0135	0,0127	0,0134	0,6761	0,676	0,6750
Morph 1.17	0,0270	0,0262	0,0265	1,1358	1,135	1,1345
Morph 1.18	0,0305	0,0317	0,0316	1,0925	1,092	1,0911
Morph 1.19	0,0332	0,0334	0,0329	1,1413	1,140	1,1395
Morph 1.20	0,0224	0,0233	0,0231	1,0145	1,014	1,0126
Morph 2.1	-0,0027	-0,0051	-0,0047	1,4615	1,4625	1,4630
Morph 2.2	0,0008	0,0010	0,0016	0,6156	0,6143	0,6141
Morph 2.3	-0,0010	-0,0009	-0,0006	0,6580	0,6578	0,6573
Morph 2.4	-0,0015	-0,0011	-0,0010	0,6054	0,6052	0,6050
Morph 2.5	-0,0009	-0,0011	-0,0011	0,5067	0,5064	0,5061
Morph 2.6	-0,0026	-0,0030	-0,0025	0,4123	0,4122	0,4120
Morph 2.7	-0,0021	-0,0024	-0,0019	1,2844	1,2841	1,2838

Morph 2.8	-0,0021	-0,0020	-0,0016	0,0743	0,7426	0,7424
Morph 2.9	-0,0024	-0,0022	-0,0019	0,8229	0,8227	0,8224
Morph 2.10	-0,0026	-0,0024	-0,0023	0,4904	0,4904	0,4890
Morph 2.11	-0,0022	-0,0022	-0,0024	0,5445	0,5442	0,5439
Morph 2.12	-0,0028	-0,0025	-0,0023	0,4127	0,4125	0,4121
Morph 2.13	-0,0030	-0,0022	-0,0028	1,1262	1,1260	1,1255
Morph 2.14	-0,0043	-0,0044	-0,0042	0,5327	0,5325	0,5322
Morph 2.15	0,0000	-0,0001	0,0000	0,3700	0,3698	0,3697
Morph 2.16	-0,0078	-0,0077	-0,0073	1,4201	1,4200	1,4201
Morph 2.17	-0,0027	-0,0026	-0,0023	1,1341	1,1341	1,1338
Morph 2.18	-0,0032	-0,0031	-0,0030	0,6665	0,6666	0,6664
Morph 2.19	-0,0025	-0,0023	-0,0021	0,4079	0,4077	0,4074
Morph 2.20	-0,0037	-0,0034	-0,0034	0,7274	0,7272	0,7272
Morph 3.1	-0,0084	-0,0084	-0,0082	1,0148	1,0146	1,0142
Morph 3.2	-0,0016	-0,0018	-0,0014	0,8188	0,8186	0,8183
Morph 3.3	-0,0037	-0,0035	-0,0035	1,0905	1,0900	1,0892
Morph 3.4	0,0027	0,0028	0,0023	0,8673	0,8671	0,8667
Morph 3.5	0,0018	0,0021	0,0014	0,6929	0,6917	0,6912
Morph 3.6	-0,0024	-0,0024	-0,0026	0,4132	0,4134	0,4134
Morph 3.7	-0,0012	-0,0014	-0,0012	0,5773	0,5771	0,5570
Morph 3.8	-0,0009	-0,0010	-0,0013	0,5685	0,5683	0,5680
Morph 3.9	0,0014	0,0018	0,0015	0,6251	0,6259	0,6256
Morph 3.10	-0,0030	-0,0033	-0,0031	0,4449	0,4447	0,4448
Morph 3.11	-0,0016	-0,0013	-0,0019	0,4792	0,4790	0,4784
Morph 3.12	-0,0011	-0,0010	-0,0010	0,6821	0,6820	0,6818
Morph 3.13	0,0004	0,0003	0,0001	1,4134	1,4132	1,4131
Morph 3.14	0,0008	0,0003	0,0003	0,8995	0,8993	0,8987
Morph 3.15	0,0000	0,0001	-0,0001	0,4673	0,4672	0,4669
Morph 3.16	-0,0010	-0,0012	-0,0011	0,6409	0,6409	0,6405
Morph 3.17	0,0002	0,0000	0,0000	0,4483	0,4480	0,4478
Morph 3.18	-0,0035	-0,0035	-0,0033	0,5778	0,5776	0,5775
Morph 3.19	-0,0031	-0,0030	-0,0030	0,4912	0,4912	0,4911
Morph 3.20	-0,0015	-0,0013	-0,0011	0,3797	0,3797	0,3794

TABLE 9. continues

Fish	Mass (PSB)	Lenght m.m	Temp C	Density Temp	Density ISB	Density PSW
Morph 1.1	0,0347	49	21,5	0,99791	1,02125	1,03522
Morph 1.2	0,0438	54	21,5	0,99791	1,01703	1,03368
Morph 1.3	0,0318	45	21,5	0,99791	1,02403	1,04393
Morph 1.4	0,0468	47	21,5	0,99791	1,02729	1,05297
Morph 1.5	0,0534	51	21,5	0,99791	1,02084	1,04656
Morph 1.6	0,0407	45	21,0	0,99802	1,02484	1,05005
Morph 1.7	0,0568	46	21,0	0,99802	1,02160	1,08533
Morph 1.8	0,0568	44	21,0	0,99802	1,02649	1,09434
Morph 1.9	x	x	x	x	x	x
Morph 1.10	0,0648	48	21,0	0,99802	1,02432	1,07137
Morph 1.11	0,0722	49	21,5	0,99791	1,02327	1,06901
Morph 1.12	0,0511	47	21,5	0,99791	1,03733	1,06722
Morph 1.13	0,0669	53	22,0	0,99780	1,02263	1,05788
Morph 1.14	0,0444	48	22,0	0,99780	1,01898	1,06097
Morph 1.15	0,0732	53	22,0	0,99780	1,02385	1,06115
Morph 1.16	0,0389	43	21,5	0,99791	1,01810	1,05886
Morph 1.17	0,0842	50	21,5	0,99791	1,02175	1,07781
Morph 1.18	0,0853	51	21,5	0,99791	1,02764	1,08244
Morph 1.19	0,0897	51	21,5	0,99791	1,02754	1,08307
Morph 1.20	0,0659	50	21,5	0,99791	1,02118	1,06729
Morph 2.1	0,0945	51	22,5	0,99768	0,99449	1,06649
Morph 2.2	0,0342	41	22,5	0,99768	1,00028	1,05645
Morph 2.3	0,0446	42	22,0	0,99780	0,99689	1,07035
Morph 2.4	0,0359	41	22,0	0,99780	0,99616	1,06067
Morph 2.5	0,0337	39	22,0	0,99780	0,99564	1,06890
Morph 2.6	0,0307	38	22,0	0,99780	0,99179	1,07804
Morph 2.7	0,0996	59	21,0	0,99802	0,99655	1,08186
Morph 2.8	0,0575	47	21,0	0,99802	0,99588	1,08171
Morph 2.9	0,0487	46	22,5	0,99768	0,99538	1,06040
Morph 2.10	0,0332	39	22,5	0,99768	0,99302	1,07026
Morph 2.11	0,0393	39	22,5	0,99768	0,99330	1,07529
Morph 2.12	0,0324	37	22,5	0,99768	0,99215	1,08271
Morph 2.13	0,0720	51	23,5	0,99744	0,99497	1,06553

Morph 2.14	0,0376	42	23,5	0,99744	0,98964	1,07318
Morph 2.15	0,0279	36	23,5	0,99744	0,99744	1,07876
Morph 2.16	0,1036	54	23,5	0,99744	0,99235	1,07584
Morph 2.17	0,0796	51	23,5	0,99744	0,99542	1,07266
Morph 2.18	0,0526	44	23,5	0,99744	0,99298	1,08281
Morph 2.19	0,0302	39	23,5	0,99744	0,99233	1,07720
Morph 2.20	0,0563	44	23,5	0,99744	0,99280	1,08104
Morph 3.1	0,0605	46	22,5	0,99768	0,98969	1,06089
Morph 3.2	0,0387	44	22,5	0,99768	0,99598	1,04715
Morph 3.3	0,0621	47	22,0	0,99780	0,99461	1,05806
Morph 3.4	0,0310	42	22,0	0,99780	1,00045	1,03477
Morph 3.5	0,0397	40	22,0	0,99780	0,99982	1,05853
Morph 3.6	0,0237	36	23,5	0,99744	0,99121	1,05803
Morph 3.7	0,0327	39	23,5	0,99744	0,99530	1,05957
Morph 3.8	0,0209	37	23,5	0,99744	0,99517	1,03550
Morph 3.9	0,0362	40	23,5	0,99744	0,99983	1,05863
Morph 3.10	0,0227	38	23,5	0,99744	0,99054	1,05102
Morph 3.11	0,0271	38	23,5	0,99744	0,99350	1,05726
Morph 3.12	0,0317	41	23,5	0,99744	0,99598	1,04602
Morph 3.13	0,0528	49	23,5	0,99744	0,99751	1,03611
Morph 3.14	0,0468	45	23,5	0,99744	0,99777	1,05217
Morph 3.15	0,0261	36	23,5	0,99744	0,99723	1,05643
Morph 3.16	0,0265	40	23,5	0,99744	0,99573	1,04044
Morph 3.17	0,0261	37	23,5	0,99744	0,99744	1,05910
Morph 3.18	0,0203	39	23,5	0,99744	0,99178	1,03374
Morph 3.19	0,0219	38	23,5	0,99744	0,99139	1,04394
Morph 3.20	0,0227	36	23,5	0,99744	0,99456	1,06084

NOTE: ISB – intact swim bladder, PSB – punctured swim bladder

TABLE 10. Mass of lateral plates of the threespine sticklebacks

Fish	Plate 8	Plate 9	Plate 10	Plate 11	Plate 12	Plate 13	Plate 14	Plate 15	Plate 16	Plate 17	Plate 18	Plate 19	Plate 20	Keel
Morph 1.1	0,873	0,726	0,639	0,486	0,467	0,416	0,339	0,288	0,219	0,178	0,154	0,096	0,081	0,736
Morph 1.2	1,239	0,868	0,801	0,763	0,753	0,593	0,570	0,482	0,278	0,329	0,191	0,186	0,141	1,144
Morph 1.3	0,635	0,719	0,587	0,468	0,360	0,339	0,319	0,263	0,190	0,175	0,133	0,076	0,060	0,829
Morph 1.4	0,627	0,503	0,544	0,435	0,424	0,404	0,307	0,327	0,232	0,215	0,163	0,153	0,107	0,856
Morph 1.5	0,903	0,874	0,794	0,757	0,689	0,545	0,507	0,412	0,357	0,274	0,235	0,197	0,167	1,470
Morph 1.6	0,588	0,420	0,358	0,313	0,269	0,261	0,195	0,198	0,145	0,117	0,087	0,085	0,059	0,742
Morph 1.7	0,593	0,389	0,376	0,317	0,291	0,268	0,219	0,205	0,163	0,121	0,095	0,099	0,067	0,802
Morph 1.8	0,532	0,395	0,376	0,312	0,276	0,292	0,181	0,157	0,124	0,111	0,107	0,091	0,080	1,253
Morph 1.9	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Morph 1.10	0,679	0,585	0,637	0,445	0,448	0,407	0,317	0,256	0,278	0,171	0,150	0,103	0,094	0,712
Morph 1.11	1,105	0,746	0,640	0,582	0,593	0,475	0,426	0,390	0,322	0,227	0,215	0,180	0,164	1,264
Morph 1.12	0,420	0,393	0,350	0,304	0,254	0,253	0,210	0,133	0,102	0,099	0,082	0,060	0,058	0,666
Morph 1.13	1,119	0,870	0,734	0,637	0,529	0,458	0,466	0,306	0,266	0,201	0,173	0,202	0,152	1,506
Morph 1.14	0,638	0,572	0,406	0,432	0,360	0,367	0,255	0,213	0,179	0,148	0,135	0,123	0,095	0,817
Morph 1.15	0,928	0,724	0,675	0,573	0,529	0,487	0,387	0,326	0,280	0,237	0,205	0,203	0,114	1,179
Morph 1.16	0,495	0,316	0,293	0,237	0,249	0,220	0,207	0,159	0,118	0,095	0,085	0,069	0,057	0,615
Morph 1.17	1,115	0,732	0,680	0,616	0,574	0,532	0,482	0,412	0,344	0,308	0,223	0,212	0,181	1,452
Morph 1.18	0,892	0,785	0,709	0,682	0,616	0,524	0,480	0,400	0,301	0,253	0,196	0,155	0,148	0,927
Morph 1.19	0,800	0,615	0,584	0,507	0,514	0,395	0,352	0,299	0,256	0,208	0,167	0,139	0,119	1,104
Morph 1.20	0,656	0,624	0,563	0,418	0,395	0,312	0,277	0,227	0,194	0,134	0,103	0,083	0,078	0,733
Morph 2.1	1,742	1,398	1,339	1,139	1,249	0,988	1,039	0,835	0,678	0,560	0,412	0,363	0,293	1,843
Morph 2.2	0,498	0,367	0,338	0,298	0,248	0,236	0,219	0,176	0,129	0,126	0,089	0,062	0,052	0,534
Morph 2.3	0,512	0,422	0,374	0,367	0,315	0,281	0,223	0,165	0,191	0,136	0,125	0,072	0,059	0,706
Morph 2.4	0,449	0,353	0,328	0,232	0,197	0,165	0,146	0,118	0,088	0,067	0,069	0,065	0,045	0,463
Morph 2.5	0,357	0,294	0,282	0,256	0,245	0,182	0,173	0,130	0,114	0,096	0,059	0,051	0,041	0,591
Morph 2.6	0,289	0,298	0,187	0,154	0,120	0,118	0,094	0,060	0,061	0,064	0,041	0,049	0,034	0,558
Morph 2.7	1,193	1,016	1,043	0,923	0,796	0,610	0,534	0,539	0,369	0,289	0,254	0,230	0,141	1,339
Morph 2.8	0,690	0,456	0,400	0,328	0,293	0,234	0,204	0,147	0,112	0,069	0,082	0,061	0,043	1,141
Morph 2.9	0,843	0,429	0,452	0,431	0,403	0,327	0,287	0,248	0,211	0,171	0,141	0,131	0,103	0,783
Morph 2.10	0,382	0,263	0,242	0,230	0,169	0,131	0,110	0,100	0,063	0,062	0,039	0,038	0,020	0,482
Morph 2.11	0,336	0,300	0,211	0,203	0,185	0,129	0,107	0,101	0,059	0,069	0,050	0,051	0,042	0,540

Morph 2.12	0,410	0,222	0,208	0,170	0,148	0,144	0,091	0,086	0,057	0,051	0,035	0,042	0,027	0,541
Morph 2.13	1,187	0,897	0,890	0,735	0,613	0,534	0,495	0,413	0,327	0,258	0,202	0,177	0,133	1,080
Morph 2.14	0,710	0,395	0,322	0,333	0,276	0,239	0,200	0,184	0,162	0,103	0,093	0,066	0,053	0,679
Morph 2.15	0,310	0,300	0,206	0,195	0,173	0,142	0,111	0,070	0,055	0,050	0,056	0,045	0,046	0,444
Morph 2.16	1,360	1,106	0,933	0,858	0,825	0,705	0,580	0,537	0,428	0,321	0,293	0,256	0,190	1,234
Morph 2.17	1,167	1,033	0,837	0,714	0,670	0,565	0,506	0,520	0,346	0,255	0,207	0,179	0,153	1,327
Morph 2.18	0,571	0,455	0,358	0,341	0,253	0,218	0,190	0,155	0,143	0,123	0,102	0,092	0,074	0,699
Morph 2.19	0,243	0,216	0,186	0,155	0,153	0,135	0,094	0,089	0,067	0,051	0,036	0,049	0,044	0,402
Morph 2.20	0,541	0,405	0,402	0,410	0,294	0,292	0,280	0,217	0,174	0,140	0,112	0,124	0,082	0,774
Morph 3.1	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.2	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.3	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.4	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.5	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.6	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.7	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.8	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.9	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.10	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.11	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.12	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.13	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.14	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.15	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.16	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.17	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.18	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.19	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Morph 3.20	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000

TABLE 11. Mass of ventral spines, plates and spines together and % of spines + plates compared to mass of fish

Fish	Mass Ventral spines	Mass plates + spines	Mass of fish	% plates + spines of body mass
Morph 1.1	21,726	33,122	961,6	0,03
Morph 1.2	24,664	41,340	1264,4	0,03
Morph 1.3	19,536	29,842	720,6	0,04
Morph 1.4	17,365	27,959	894,0	0,03
Morph 1.5	24,251	40,613	1147,5	0,04
Morph 1.6	15,850	23,524	820,5	0,03
Morph 1.7	16,356	24,366	705,3	0,03
Morph 1.8	14,894	23,468	644,6	0,04
	x	x	x	x
Morph 1.10	18,916	29,480	945,4	0,03
Morph 1.11	24,700	39,358	1084,3	0,04
Morph 1.12	16,145	22,913	785,9	0,03
Morph 1.13	24,592	39,830	1176,6	0,03
Morph 1.14	16,605	26,085	744,9	0,04
Morph 1.15	22,907	36,601	1224,7	0,03
Morph 1.16	12,201	18,631	675,0	0,03
Morph 1.17	27,090	42,816	1134,5	0,04
Morph 1.18	22,730	36,866	1091,1	0,03
Morph 1.19	20,212	32,330	1139,5	0,03
Morph 1.20	18,936	28,530	1012,6	0,03
Morph 2.1	26,435	54,191	1463,0	0,04
Morph 2.2	14,621	21,365	614,1	0,03
Morph 2.3	14,213	22,109	657,3	0,03
Morph 2.4	12,088	17,658	605,0	0,03
Morph 2.5	10,756	16,498	506,1	0,03
Morph 2.6	11,279	15,533	412,0	0,04
Morph 2.7	28,379	46,931	1283,8	0,04
Morph 2.8	14,404	22,924	742,4	0,03
Morph 2.9	15,754	25,674	822,4	0,03
Morph 2.10	11,390	16,052	489,0	0,03
Morph 2.11	9,832	14,598	543,9	0,03

Morph 2.12	8,941	13,405	412,1	0,03
Morph 2.13	19,730	35,612	1125,5	0,03
Morph 2.14	10,064	17,694	532,2	0,03
Morph 2.15	10,022	14,428	369,7	0,04
Morph 2.16	30,764	50,016	1420,1	0,04
Morph 2.17	22,016	38,974	1133,8	0,03
Morph 2.18	13,704	21,252	666,4	0,03
Morph 2.19	8,415	12,255	407,4	0,03
Morph 2.20	18,012	26,506	727,2	0,04
Morph 3.1	5,170	5,170	1014,2	0,01
Morph 3.2	5,445	5,445	818,3	0,01
Morph 3.3	6,987	6,987	1089,2	0,01
Morph 3.4	5,423	5,423	866,7	0,01
Morph 3.5	4,693	4,693	691,2	0,01
Morph 3.6	3,341	3,341	413,4	0,01
Morph 3.7	3,907	3,907	557,0	0,01
Morph 3.8	3,915	3,915	568,0	0,01
Morph 3.9	3,907	3,907	625,6	0,01
Morph 3.10	3,253	3,253	444,8	0,01
Morph 3.11	2,790	2,790	478,4	0,01
Morph 3.12	4,168	4,168	681,8	0,01
Morph 3.13	7,815	7,815	1413,1	0,01
Morph 3.14	5,535	5,535	898,7	0,01
Morph 3.15	3,196	3,196	466,9	0,01
Morph 3.16	4,372	4,372	640,5	0,01
Morph 3.17	3,446	3,446	447,8	0,01
Morph 3.18	3,495	3,495	577,5	0,01
Morph 3.19	3,516	3,516	491,1	0,01
Morph 3.20	3,297	3,297	379,4	0,01

APPENDIX II

TABLE FOR SARTORIUS YDK 01

TABLE 12. Table for Sartorius YDK 01; density of H_20 at Temperature T (in °C)

Temperature	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
10.	0,99973	0,99972	0,99971	0,99970	0,99969	0,99968	0,99967	0,99966	0,99965	0,99964
11.	0,99963	0,99962	0,99961	0,99960	0,99959	0,99958	0,99957	0,99956	0,99955	0,99954
12.	0,99953	0,99951	0,99950	0,99949	0,99948	0,99947	0,99946	0,99944	0,99943	0,99942
13.	0,99941	0,99939	0,99938	0,99937	0,99935	0,99934	0,99933	0,99931	0,99930	0,99929
14.	0,99927	0,99926	0,99924	0,99923	0,99922	0,99920	0,99919	0,99917	0,99916	0,99914
15.	0,99913	0,99911	0,99910	0,99908	0,99907	0,99905	0,99904	0,99902	0,99900	0,99899
16.	0,99897	0,99896	0,99894	0,99892	0,99891	0,99889	0,99887	0,99885	0,99884	0,99882
17.	0,99880	0,99879	0,99877	0,99875	0,99873	0,99871	0,99870	0,99868	0,99866	0,99864
18.	0,99862	0,99860	0,99859	0,99857	0,99855	0,99853	0,99851	0,99849	0,99847	0,99845
19.	0,99843	0,99841	0,99839	0,99837	0,99835	0,99833	0,99831	0,99829	0,99827	0,99825
20.	0,99823	0,99821	0,99819	0,99817	0,99815	0,99813	0,99811	0,99808	0,99806	0,99804
21.	0,99802	0,99800	0,99798	0,99795	0,99793	0,99791	0,99789	0,99786	0,99784	0,99782
22.	0,99780	0,99777	0,99775	0,99773	0,99771	0,99768	0,99766	0,99764	0,99761	0,99759
23.	0,99756	0,99754	0,99752	0,99749	0,99747	0,99744	0,99742	0,99740	0,99737	0,99735
24.	0,99732	0,99730	0,99727	0,99725	0,99722	0,99720	0,99717	0,99715	0,99712	0,99710
25.	0,99707	0,99704	0,99702	0,99699	0,99697	0,99694	0,99691	0,99689	0,99686	0,99684
26.	0,99681	0,99678	0,99676	0,99673	0,99670	0,99668	0,99665	0,99662	0,99659	0,99657
27.	0,99654	0,99651	0,99648	0,99646	0,99643	0,99640	0,99637	0,99634	0,99632	0,99629
28.	0,99626	0,99623	0,99620	0,99617	0,99614	0,99612	0,99609	0,99606	0,99603	0,99600
29.	0,99597	0,99594	0,99591	0,99588	0,99585	0,99582	0,99579	0,99576	0,99573	0,99570
30.	0,99567	0,99564	0,99561	0,99558	0,99555	0,99552	0,99549	0,99546	0,99543	0,99540

APPENDIX III

TABLES FOR STATISTICS

```
T-TEST
GROUPS = pop(1 2)
/MISSING = ANALYSIS
/VARIABLES = swimbl
/CRITERIA = CI(.95) .
```

T-Test

[DataSet1] \\helix.klient.uib.no\biohome\tkl081\Gasterosteus aculeatus\Buyoancy\buoyancy1.sav

Group Statistics

	pop	N	Mean	Std. Deviation	Std. Error Mean
swimbl	1	19	4,217826	1,2110285	,2778290
	2	20	7,926900	,9567576	,2139375

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
swimbl	Equal variances assumed	1,144	,292	-10,642	37	,000	-3,709073	,3485233	-4,4152490	-3,0028986
	Equal variances not assumed			-10,578	34,263	,000	-3,709073	,3506540	-4,4214873	-2,9966603

NOTE: pop 1 – marine completely plated sticklebacks; pop 2 – freshwater completely plated stickleback; pop 3 – freshwater low plated sticklebacks (goes for all tables in Appendix III)

```
T-TEST
GROUPS = pop(1 3)
/MISSING = ANALYSIS
/VARIABLES = swimbl
/CRITERIA = CI(.95) .
```

T-Test

```
[DataSet1] \\helix.klient.uib.no\biohome\tkl081\Gasterosteus
aculeatus\Buyoancy\buoyancy1.sav
```

Group Statistics

	pop	N	Mean	Std. Deviation	Std. Error Mean
swimbl	1	19	4,217826	1,2110285	,2778290
	3	20	5,529047	1,0655089	,2382550

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
swimbl	Equal variances assumed	,329	,570	-3,595	37	,001	-1,3112208	,3647726	-2,0503203	-,5721213
	Equal variances not assumed			-3,583	35,84	,001	-1,3112208	,3659978	-2,0536108	-,5688309

```
T-TEST
GROUPS = pop(2 3)
/MISSING = ANALYSIS
/VARIABLES = swimbl
/CRITERIA = CI(.95) .
```

T-Test

```
[DataSet1] \\helix.klient.uib.no\biohome\tkl081\Gasterosteus
aculeatus\Buyoancy\buoyancy1.sav
```

Group Statistics

	pop	N	Mean	Std. Deviation	Std. Error Mean
swimbl	2	20	7,926900	,9567576	,2139375
	3	20	5,529047	1,0655089	,2382550

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
swimbl	Equal variances assumed	,266	,609	7,488	38	,000	2,3978530	,3202104	1,7496209	3,0460851
	Equal variances not assumed			7,488	37,56	,000	2,3978530	,3202104	1,7493759	3,0463300

T-Test

[DataSet1] \\helix.klient.uib.no\biohome\tkl081\Gasterosteus aculeatus\Buyoancy\buoyancy1.sav

Group Statistics

	pop	N	Mean	Std. Deviation	Std. Error Mean
Buoy	1	19	,000657	,0042914	,0009845
	2	20	,005339	,0024296	,0005433

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Buoy	Equal variances assumed	1,456	,235	-4,221	37	,000	-,0046821	,0011093	-,0069298	-,0024344
	Equal variances not assumed			-4,164	28,158	,000	-,0046821	,0011245	-,0069849	-,0023793

T-Test

Group Statistics

	pop	N	Mean	Std. Deviation	Std. Error Mean
Buoy	1	19	,000657	,0042914	,0009845
	3	20	,004247	,0032784	,0007331

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Buoy	Equal variances assumed	,172	,680	-2,945	37	,006	-,0035900	,0012190	-,0060599	-,0011201
	Equal variances not assumed			-2,925	33,68	,006	-,0035900	,0012275	-,0060854	-,0010946

T-Test

Group Statistics

	pop	N	Mean	Std. Deviation	Std. Error Mean
Buoy	2	20	,005339	,0024296	,0005433
	3	20	,004247	,0032784	,0007331

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Buoy	Equal variances assumed	1,171	,286	1,197	38	,239	,0010921	,0009124	-,0007551	,0029392
	Equal variances not assumed			1,197	35,03	,239	,0010921	,0009124	-,0007602	,0029444

T-Test

Group Statistics

	pop	N	Mean	Std. Deviation	Std. Error Mean
Tissued	1	19	1,066775	,0134357	,0030824
	2	20	1,072972	,0079933	,0017873

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Tissued	Equal variances assumed	5,431	,025	-1,761	37	,086	-,0061973	,0035186	-,0133266	,0009321
	Equal variances not assumed			-1,739	29,03	,093	-,0061973	,0035631	-,0134843	,0010897

T-Test

Group Statistics

	pop	N	Mean	Std. Deviation	Std. Error Mean
Tissued	1	19	1,066775	,0134357	,0030824
	3	20	1,050311	,0097517	,0021806

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Tissued	Equal variances assumed	1,701	,200	4,396	37	,000	,0164641	,0037450	,0088760	,0240521
	Equal variances not assumed			4,361	32,75	,000	,0164641	,0037757	,0087802	,0241480

T-Test

Group Statistics

	pop	N	Mean	Std. Deviation	Std. Error Mean
Tissued	2	20	1,072972	,0079933	,0017873
	3	20	1,050311	,0097517	,0021806

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Tissued	Equal variances assumed	2,007	,165	8,037	38	,000	,0226613	,0028195	,0169536	,0283691
	Equal variances not assumed			8,037	36,591	,000	,0226613	,0028195	,0169464	,0283763

Oneway

Descriptives

Fishd

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	19	1,023643	,0042914	,0009845	1,021575	1,025712	1,0176	1,0365
2	20	,994261	,0024296	,0005433	,993124	,995398	,9895	,9995
3	20	,995353	,0032784	,0007331	,993819	,996888	,9896	1,0008
Total	59	1,004093	,0140018	,0018229	1,000444	1,007742	,9895	1,0365

ANOVA

Fishd

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,011	2	,005	463,444	,000
Within Groups	,001	56	,000		
Total	,011	58			

T-Test

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Fishd	19	1,023643	,0042914	,0009845

One-Sample Test

	Test Value = 1.024					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Fishd	-,362	18	,721	-,0003568	-,002425	,001712

T-Test

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Fishd	20	,994261	,0024296	,0005433

One-Sample Test

	Test Value = 1.0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Fishd	-10,563	19	,000	-,0057388	-,006876	-,004602

T-Test

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Fishd	20	,995353	,0032784	,0007331

One-Sample Test

	Test Value = 1.0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Fishd	-6,339	19	,000	-,0046468	-,006181	-,003112