The effect of testosterone injections on aggression and begging behaviour of black headed gull chicks (*Larus ridibundus*)

L.M. van Zomeren
april 2009
supervised by Giuseppe Boncoraglio and Ton G. G. Groothuis.

Abstract

Although testosterone plays an important role in complex social behaviour, the effects of this hormone on developing avian chicks remain rather unclear. Previous research on pied flycatcher chicks shows an increase in begging behaviour after experimentally increasing testosterone plasma levels in the short term, while implantation with testosterone in black headed gull chicks results in a decrease in begging behaviour. This opposite role of testosterone in these two avian species could be an intriguing species-specific difference, but could also be the result of the different experimental methodology adopted by the two studies. This study manipulates plasma testosterone levels of black headed gull chicks by means of injections, raising the circulating hormone levels of the chicks only in the short-term (i.e. a few hours). Injections of testosterone resulted in a decrease in begging behaviour, an increase in aggression and an increase in sibling competition. Therefore it can be concluded that the begging intensity of black headed gull chicks is not decreased by testosterone, which is the opposite of the effect found in pied flycatcher chicks.

Introduction

Gonadal steroids play a very important role in the regulation of complex behaviour in vertebrates. Much research has been done on the effects of various hormones on adults, while only a few studies have been done on young dependent offspring. In precocial and semi precocial avian species social behaviour in this stage of development is very important for survival. In a semi-precocial species like the black-headed gull (*Larus ridibundus*) the most important social behaviours are begging behaviour towards the parents to solicit food and territorial behaviour towards non-sibling (Groothuis, 1989). The begging behaviour consists of various conspicuous displays and calls and it serves to solicit food from the parents. The displays are head pumping, in which the chick moves its head up and down, begging calls, which are frequent high-pitched sounds and begging pecks, which are quick non-aggressive pecks towards the bill of the parent. The territorial behaviours consist of threat postures and calls and also actual pecking and fighting. The most common treat postures are the oblique and the choking display. The oblique display is a posture were the chicks is standing up tall with the head raised and wing somewhat open and is typical for older chicks. The choking display is a posture were the chick lies down and pecks into the ground. Loud alarm calls are uttered in all aggressive displays. These behaviours are displayed to protect the food brought to the nest by the parents from non-siblings in the colony (Groothuis, 1989). It is surprising that up to now only a few studies have tried to unravel the hormones behind these behaviours. Recent evidence suggests a strong role for steroid hormones, mainly testosterone, in the regulation of social behaviour of chicks. Correlative studies on passerines, for example on canaries (*Serinus canaria*), show a correlation between circulating testosterone levels and begging intensity (Buchanan et al., 2007). Previous research on black headed gull chicks involved implantation of testosterone capsules that constantly released testosterone into the blood throughout the whole period of the experiment. These chicks showed more aggressive behaviour than their controls as well as a reduction in begging behaviour (Groothuis and Ros,

2005). These findings are contradictory to research performed in pied flycatchers (*Ficedula hypoleuca*), in which begging behaviour was increased after administrating testosterone (Goodship and Buchanan, 2007). The difference between these studies could be because of a species specific role for testosterone in the regulation of begging behaviour. However, these studies differ not only in species but also in way of hormone administration. Flycatcher chicks were given testosterone orally just before performing the begging tests, while the black headed gull chicks were implanted with crystalline testosterone pellets. Therefore, the testosterone plasma concentration of the canaries therefore shows a steep and short peak, while the black headed gull chicks perceive a long term, constant elevated testosterone plasma level. The opposition in the effect of testosterone on the begging behaviour of these two species could be related to the way testosterone was administered. A continuous elevated level of testosterone in the blood could have an entirely different effect compared to a short rise in testosterone plasma levels. If the way of administering the testosterone is not responsible for the difference in the effect of this hormone, then the altricial flycatcher might have an opposite response to testosterone as the semi-precocial black headed gull. This difference in the two species could reflect a very interesting divergent evolutionary path for the function of testosterone. To find more information about this interesting possibility, black headed gulls are injected with testosterone to reproduce short term rises in testosterone plasma levels in this study.
Corticosterone is also a hormone that could be involved in regulating begging behaviour in young birds (e.g. Love *et al*., 2003; Kitaysky *et al*., 2001). In black-legged kittiwake (*Rissa tridactyla)* chicks implantation of a silastic tube filled with crystallized corticosterone resulted in an increase of begging frequency (Kitaysky *et al*., 2001). Injections with corticosterone dissolved in sesame oil did not result in elevated corticosterone plasma levels in a pilot done on black headed gull chicks. However, injections with pure sesame oil did result in an increase in circulating corticosterone plasma levels. In this study corticosterone levels are therefore manipulated by injection with oil in black headed gull chicks. It is expected that increasing the circulating corticosterone levels will result in a higher begging intensity.
This study should give insight in the hormonal control of begging behaviour, aggression and sibling competition in black headed gull chicks.

Material and Methods

Animals
Three day old first-hatched *Larus ridibundus* chicks were collected in a natural breeding colony in the North of the Netherlands. Groups of three matched for weight where kept in 85 x 75 x 85 cm wire mesh cages with sawdust bedding. Chicks from different cages could not see each other. Food and water was provided *ad libitum* and daily refreshed. Food consisted of moistened pellets used for trout farming (Trouvit, Trouw, Gent). Five times a day the chicks were fed by hand with moistened trout pellets and pieces of fish presented by tweezers. The room was kept at a temperature between 22 – 28 °C at a LD cycle of 14:10.
Age of the chicks is presented as days from birth, with day 3 being the day of capturing.

Hormone treatment
On days 14, 17 and 20 chicks were treated with hormones. In a pilot study, injections with corticosterone did not affect corticosterone plasma levels. However, when injecting oil corticosterone plasma levels did rise to about 170% of normal level. Therefore, for this study injections with oil are used to increase the circulating levels of corticosterone.
For every cage one randomly selected chick would be injected with testosterone dissolved in sterile sesame oil (i.e. increase of testosterone and corticosterone), one with sesame oil only

(i.e. increase of corticosterone), and one would be unhandled. In two cages one chick died before reaching the age of 14 days, in these cases the two chicks that where left where treated with oil and testosterone.
Testosterone was dissolved in sterile sesame oil in a concentration of 100 μg/ml. Chicks where injected intraperitoneally with 50 μl testosterone-oil solution or pure sesame oil. Thirty minutes after hormone treatment tests where performed. To assess the effectiveness of this method, a pilot was performed the year before. In this pilot, testosterone injections produced a increase of testosterone in blood plasma to about 100% of normal baseline levels (0.20 ng/ml) half an hour after injection, while oil injections increased circulating corticosterone levels with about 70 % of baseline levels (2.5 ng/ml), being however well within the natural range of variation in plasma concentration of both hormones for this species.

Tests
Behavioural tests where performed according to the plan depicted in Table 1. All tests are referred to as described in this table.

Table 1. Plan of behavioural tests including test designation.

Test	Referred to as	Day	Food deprived	Treated
Begging	Baseline begging	12 and 13	yes	no
Begging	Begging after treatment	14	yes	yes
Fear	Fear score	16 and 20	no	no, yes
Hunger	Hunger score	16 and 20	no	no, yes
Aggression	Aggression score	17	yes	yes
Competition	Competition score	20	yes	yes

Begging test
When opening the door of the cage, the behaviour of the chicks was observed for 10 seconds. A focal animal was chosen at random and observed, after 10 seconds the next random focal was observed. The number of head pumps and begging calls each chick performed was scored. Also the posture of the chick was observed and ranked according to the following ranking system: 5 = Head pumping, constantly uttering calls, beak open; 4 = Head pumping, calls, beak mostly closed; 3 = Calling continuously, no pumping; 2 = Watching, some calls; 1 = Watching only; 0 = Not interested, not watching. This is referred to as 'begging posture'. After this the chicks where presented with empty tweezers to provoke begging pecks naturally performed towards the bill of a parent. The number of pecks in 10 seconds was scored for each chick, using the same order of chicks as in the begging posture. This is referred to as 'Begging pecks'.

Aggression test
At day 17 the aggression test was performed. A stuffed adult gull in threatening posture was placed inside the cage of the chicks. During 30 seconds the number of aggressive pecks towards this model was scored for each chick. Also the behaviour was observed every 6 seconds and ranked according to the following ranking system: 3 = Pecking at the model; 2 = Oblique posture; 1 = Choking display; 0 = Freezing, no sound; -1 = Begging towards the model. Individual scores were added up over the whole 30 s. trial and the sum was computed. This test was repeated twice and the average individual sum is referred to as 'aggression score'.

Competition test

After food depriving the chicks for 3 hours, tweezers holding a piece of fish at equal distance between the chicks where introduced into the cage. The chick that swallowed the fish was notated and this protocol was repeated ten times.
From this a feeding rank score was computed. Each trial was given a ranking number, trial 1 being the first piece of fish presented, 10 being the last piece of fish. For each chick the average trial number of which it swallowed the fish was computed. This is referred to as 'Feeding rank score'. A low number means a more competitive chick, because it will feed before its cage-mates until somewhat satiated.
The proportion of total food each chick swallowed was expressed as a number between 0 and 1 and computed by an arcsin square root transformation; Number of items eaten (transformed value) = arcsin (√((number of food items eaten)/total food items)).
The total number of pieces of fish eaten was also scored and is referred to as 'items eaten'.
The competition test was done twice, starting the second test shortly after the first, however only the first test is used because of a satiation effect in the second test.

Individual behavioural differences
When feeding the chicks, individual variation in hunger level, fear of the experimenter and begging intensity was observed between the chicks. This was constant over different feeding bouts (repeatability tests, details not shown). To be able to correct for these parameters, the level of hunger and of fear was measured once without hormone treatment and once 30 minutes after hormone treatment.
Hunger level was scored using the following criteria: 4 = Eating > 20 pellets from tweezers; 3 = Many pellets eaten, but not more than 20; 2 = Watching closely, some pellets; 1 = Only fish eaten; 0 = Nothing eaten. This is referred to as 'hunger score'.
Fear intensity was scored using the following criteria: 4 = Screaming in corner; 3 = Standing far away, alarm calls; 2 = Watching from a distance, getting some food; 1 = Standing close, back away after feeding; 0 = Begging closely, no alarm calls, staying close until experimenter leaves. This is referred to as 'Fear score'.

Statistics
All statistical tests where performed with SPSS. Data were analyzed using One-way ANOVA and General Linear Model ANOVA approaches, sometimes with Sidak correction for multiple testing. Results are considered significant when $p < 0,05$. Significant results are marked with (*).

Results

Group assortment with respect to the assignation of individual treatment
Assigning the chicks to the hormone treatment was found to be unbiased. Chicks did not differ across groups with respect to body mass, tarsus length and begging behaviour prior to hormone treatment (One-way ANOVA, $p > 0.181$ in all cases).

Effect of hormones on begging behaviour
The treatment with testosterone and oil had a significant effect on some components of the begging behaviour of the chicks. Treatment had a significant influence on the number of begging pecks towards the spoon (Table 2; Figure 1), while it did not affect head pumping, begging posture or calling behaviour.

Table 2. Effect of hormone treatment on begging behaviour of 14-day old chicks. One-way ANOVA.

	Df	F	Sig.

Head pumps	Between Groups	2	0,460	0,635
	Within Groups	31		
Posture	Between Groups	2	0,001	0,999
	Within Groups	31		
Pecks	Between Groups	2	4,413	0,021 (*)
	Within Groups	31		
Calls	Between Groups	2	0,148	0,863
	Within Groups	31		

When focussing on the effects of the different treatments (testosterone, oil and unhandled control) on the number of begging pecks, testosterone injected chicks beg significantly less than oil injected birds (Table 3). There is an indication that oil injected birds beg more than control birds, although this is only a trend (Table 3).

Table 3. The effect of the different treatments on begging pecks of 14-day old chicks. One-way ANOVA with Sidak correction for multiple testing.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Testosterone	Oil	-4,583 (*)	1,617	0,024	-8,66	-,50
	Control	-,967	1,696	0,922	-5,25	3,31
Oil	Testosterone	4,583 (*)	1,617	0,024	,50	8,66
	Control	3,617	1,696	0,118	-,66	7,90
Control	Testosterone	,967	1,696	0,922	-3,31	5,25
	Oil	-3,617	1,696	0,118	-7,90	,66

Correcting the data for cage (fixed factor), tarsus length or baseline begging behaviour (covariates) had no effect. Correcting for body mass at day 12, together with the interaction between testosterone treatment and body mass, did increase the total variance explained by the model (Table 4).

Table 4. The effect of the different treatments on begging pecks at day 14, corrected for mass and treatment*mass. General Linear Model ANOVA.

	df	F	Sig.
Treatment	2	9,675	0,001 (*)
Mass at day 12	1	11,070	0,002 (*)
Treatment * mass at day 12	2	6,953	0,004 (*)

When doing a pairwise comparison on the corrected data, testosterone injected chicks begged significantly less than oil injected birds. Oil birds begged significantly more compared to all other groups (Table 5). Figure 1 shows this relationship and the effect of chick body mass on begging pecks across experimental groups more clearly.

Table 5. The effect of the different treatments on begging calls of 14-day old chicks corrected for body mass and treatment*mass. General Linear Model Anova with Sidak correction for multiple testing.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Testosterone	Oil	-5,665 (*)	1,311	0,001	-8,995	-2,336
	Control	-,582	1,413	0,968	-4,170	3,006
Oil	Testosterone	5,665 (*)	1,311	0,001	2,336	8,995
	Control	5,083 (*)	1,433	0,004	1,445	8,722

Control	Testosterone Oil	,582 -5,083 (*)	1,413 1,433	0,968 0,004	-3,006 -8,722	4,170 -1,445

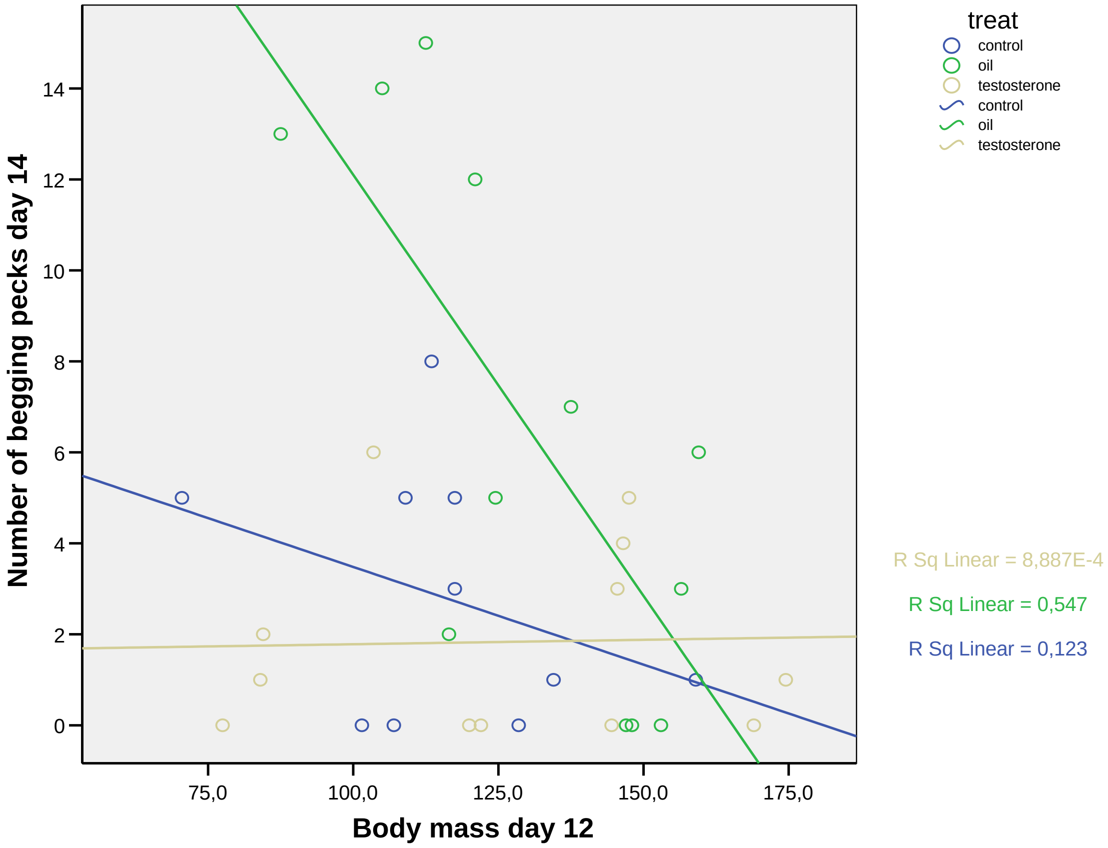

Figure 1. The effect of treatment and body mass on begging pecks performed by 14-day old black headed gull chicks. Control and oil differ significantly, as well as testosterone and oil treatment.

Effect of hormones on aggression

When testing with a one-way ANOVA, the effect of treatment on the aggression score was not significant. However, it showed a trend for testosterone chicks being more aggressive than the other ones ($F = 2{,}453$; $df = 2, 31$; $p = 0{,}103$).

Correcting for day 12 tarsus length and body mass had no effect. Correcting for fear and hunger score at day 16 and cage did have a significant effect (Table 6).

Table 6. The effect of treatment on aggression score at day 17, corrected for fear score, hunger score and cage. General Linear Model ANOVA.

	df	F	Sig.
Treatment	2	5,802	0,011 (*)
Fear score day 16	1	4,212	0,055
Hunger score day 16	1	7,223	0,015 (*)
Cage	11	2,913	0,021 (*)

Pairwise comparisons of corrected data show that testosterone injected chicks have a higher aggression score than both control and oil injected chicks (Table 7). Oil injected and control birds do not differ in aggression score.

Table 7. Pairwise comparisons of the effect of hormone treatment on aggression score at day 17, corrected for fear score, hunger score and cage. General Linear Model ANOVA with Sidak correction for multiple testing

(I)	(J)	Mean	Std. Error	Sig.	95% Confidence Interval

Treatment	Treatment	Difference (I-J)			Lower Bound	Upper Bound
Testosterone	Oil	3,102 (*)	1,145	0,042	,091	6,114
	Control	3,694 (*)	1,193	0,019	,556	6,831
Oil	Testosterone	-3,102 (*)	1,145	0,042	-6,114	-,091
	Control	,592	1,202	0,949	-2,572	3,755
Control	Testosterone	-3,694 (*)	1,193	0,019	-6,831	-,556
	Oil	-,592	1,202	0,949	-3,755	2,572

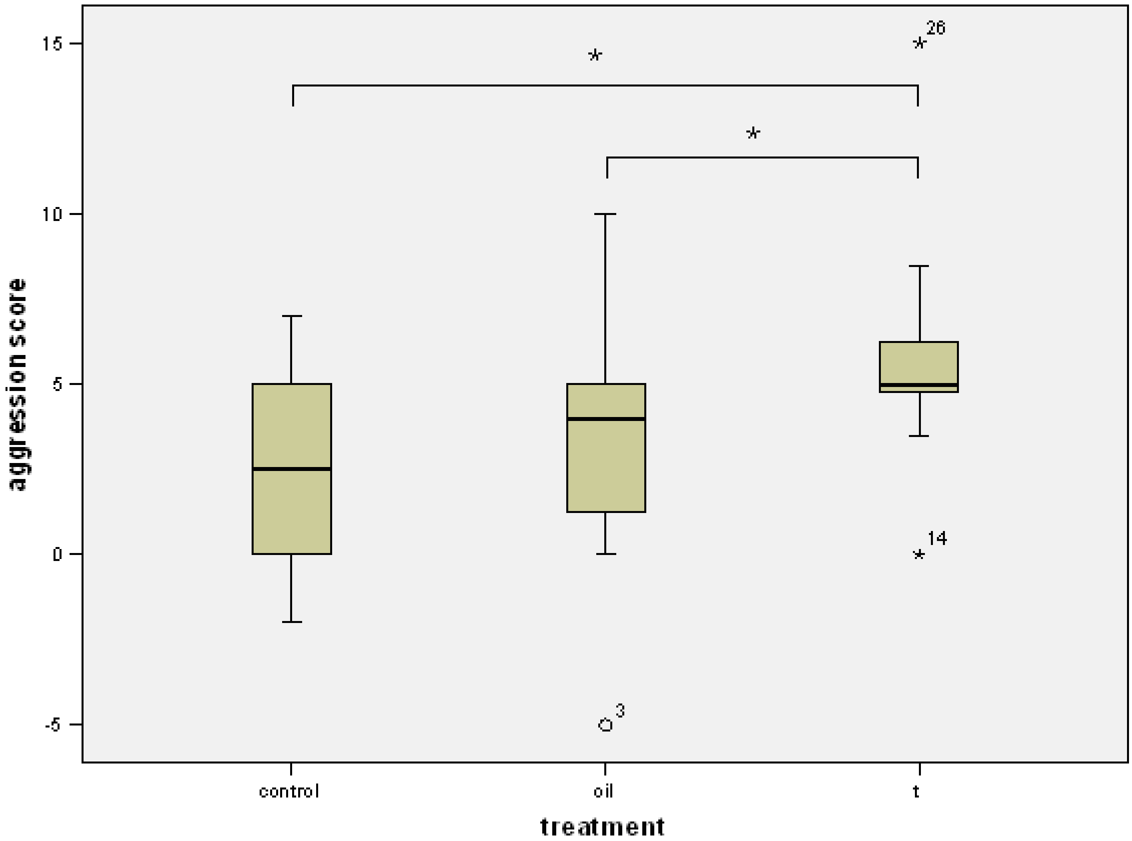

Figure 2. The effect of the different treatments on the aggression score at day 17.

The same analysis performed on the number of aggressive pecks to the stuffed model did not show any significant result (details not shown).

Effect of hormones on sibling competition

When testing with a one-way ANOVA the total items eaten (arcsin square root-transformed values) by each chick during the trials, no significant effect of treatment was found (data not shown). Conversely, average rank of the feeding trials, which focuses on the early or late feeding of individual chicks compared to the cage-mates, does show a significant effect of treatment (Table 8). In a pairwise comparison the testosterone injected chicks have a significantly lower feeding rank score (p=0.04; mean difference = -1.66406; St. Error = 0.063354) than control chicks (Figure 3). This means testosterone injected chicks collect food significantly earlier than the other chicks.

Table 8. Effect of treatment on food competition in 20-day old food deprived chicks. One way ANOVA.

		df	F	Sig.
Portion of items eaten	Between Groups	2	1,008	0,377

		df	F	Sig.
	Within Groups	31		
Feeding rank score	Between Groups	2	3,782	0,035 (*)
	Within Groups	29		

Correcting for cage, tarsus length, fear score and hunger score at the same day, or any interaction had no effect. Correcting for body mass shows a positive trend and was therefore kept in the model (Table 9).

Table 9. Effect of treatment on feeding rank in the first trial in 20-day old chicks, corrected for body mass measured at the same day. One way ANOVA.

	df	F	Sig.
Treatment	2	5,270	0,011 (*)
Body mass	1	3,133	0,088

A pairwise comparison shows that testosterone injected chicks collected food earlier than control chicks during the competition test (Table 10). A trend can be observed in which the testosterone injected chicks also collected food earlier than oil injected chicks, although this is not significant at the 0,05 level.

Table 10. Pairwise comparison of the effect of treatment on feeding rank in the first trial in 20-day old food deprived chicks, corrected for body mass. GLM ANOVA with Sidak correction for multiple testing.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Testosterone	Oil	-0,133	0,058	0,082	-,279	,013
	Control	-0,201 (*)	0,064	0,012	-,364	-,038
Oil	Testosterone	0,133	0,058	0,082	-,013	,279
	Control	-0,068	0,061	0,614	-,223	,086
Control	Testosterone	0,201 (*)	0,064	0,012	,038	,364
	Oil	0,068	0,061	0,614	-,086	,223

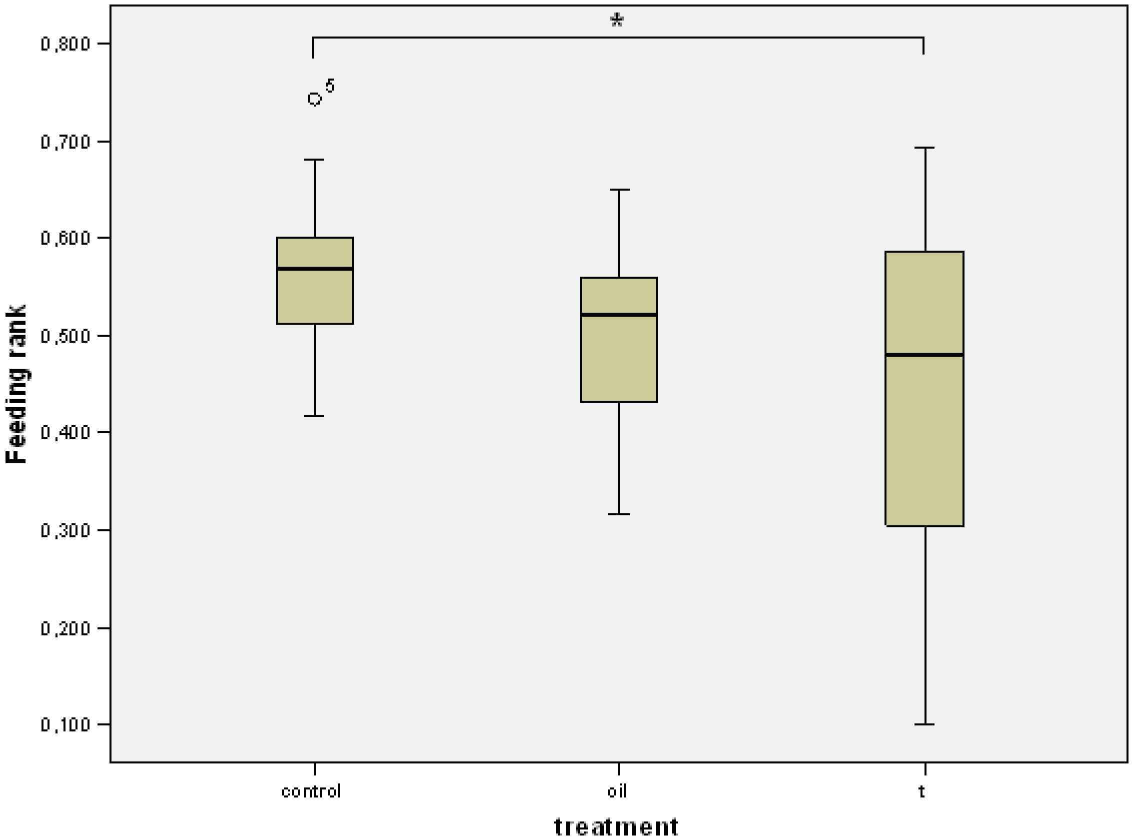

Figure 3. The effect of treatment on feeding rank of 20-day old black headed gull chicks. A low feeding rank means early collection on food.

Conclusion and discussion

Begging behaviour was suppressed by injection of testosterone, while aggressive behaviour was increased. This confirms the results of previous research done by Groothuis and Ros (2005) in which black headed gull chicks were implanted with crystallized testosterone.
The response of black headed gull chicks to rises in testosterone blood plasma concentration is thus opposite of the effect found in pied flycatcher chicks (Goodship and Buchanan, 2007). Although the method of administration of the hormone varied (oral administration vs. injections), the effect on the plasma levels seems to be the same (based on pilot data; will later be confirmed by analysis of blood samples taken during this experiment). Therefore it can be stated that these two species have an opposite role of testosterone in begging behaviour. Pied flycatchers increase begging behaviour when testosterone blood plasma concentration is elevated, while black headed gull chicks decrease begging behaviour when testosterone blood plasma concentration is elevated.
As stated previously, injections with oil have been found to increase corticosterone levels in the blood plasma of black headed gull chicks (pilot study 2008). The trend found on the decrease of aggressive behaviour and the significant increase of begging behaviour in oil injected chicks could be due to this rise in corticosterone. In black-legged kittiwake chicks (*Rissa tridactyla*) corticosterone is also responsible for an increase in begging behaviour (Kitaysky *et al*., 2001). It is very well possible that corticosterone is also the hormone responsible for begging behaviour in black headed gull chicks. In future research the role of corticosterone could be determined in a similar way as described in this study.

The opposite effect of testosterone in pied flycatchers and black headed gull raises some very interesting questions. Of course it would be very interesting to conduct similar tests as performed in this study in other bird species, both altricial and (semi)precocial, to determine the species-specific challenges that determine the regulation of begging behaviour.

References

Buchanan, K.L., Goldsmith, A.R., Hinde, C.A., Griffith, S.C., Kilner, R.M. *Does testosterone mediate the trade-off between nestling begging and growth in the canary (Serinus canaria)?* (2007) Hormones and Behavior 52, 664–671.

Eising, C.M., Groothuis, T.G.G. *Yolk androgens enhance begging behavior in black-headed-gull chicks*. (2003) Anim. Behav. 66, 1027–1034.

Goodship, N.M., Buchanan, K.L. (2007) *Nestling testosterone controls begging behaviour in the pied flycatcher, Ficedula hypoleuca* Hormones and Behavior 52 454–460

Groothuis, T.G.G. (1989) *On the ontogeny of display behaviour in the black-headed gull: II. Causal links between the development of aggression, fear and display behaviour: emancipation reconsidered*. Behaviour 110**,** 161–204.

Groothuis, T.G.G., Ros, A.F.H. *The hormonal control of begging and early aggressive behavior: Experiments in black-headed gull chicks* (2005) Hormones and Behavior 48, 207 – 215.

Kitaysky, A.S., Wingfield, J.C., Piatt, J.F. *Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake* (2001) Behavioral Ecology Vol. 12 No. 5: 619–625.

Love, O.P., Bird, D.M., Shutt, L.J. (2003) *Plasma corticosterone in American kestrel siblings: effects of age, hatching order, and hatching asynchrony* Hormones and Behavior 43 480–488