

Our Logo: The “Phaistos Disk” one of the oldest transportable text to be found to date, (still to be deciphered), has been excavated from the Phaistos Palace in Crete, and dates back to the Minoan Civilization (2nd millennium B.C.)



***12th International Symposium on Reproductive Physiology of Fish,
Crete, Greece, 15-19 May 2023***
“Reproductive science for aquaculture production and conservation”

5th Circular Announcement (4/2023)

Dear friends,

Finally, the time has arrived for the last preparations for the symposium. For a “real time” update on all issues related to the symposium, please visit and “**stay tuned**” to our website:

<https://12isrpf.weebly.com>

We provide you here with some useful information and instructions for the symposium.

Oral Presentations

A presenter’s desk will be available near the registration desk. All presenters should submit their Oral presentation to the organizers, **the day before the presentation**. Bring your presentation (in Microsoft Powerpoint) saved on a USB memory stick. If you are using an Apple Mac computer to prepare your presentation at home, make sure that it runs well on a Windows-based computer, especially if you have videos or soundtracks.

The file should be labeled as “Oral **X**_Lastname_Session **Z**”, where:

X = sequence number of the oral presentation, according to the Symposium Program,

Lastname = the last name of the presenter, and **Z** = Special session number

You should check your presentation in the presenter’s desk computer, and make sure that all slides show well. All presentations will be loaded on the projection computer **the day before** each Special Session, and no modifications can be made during the day.

See later, for suggestions/advice on preparing your oral presentation.

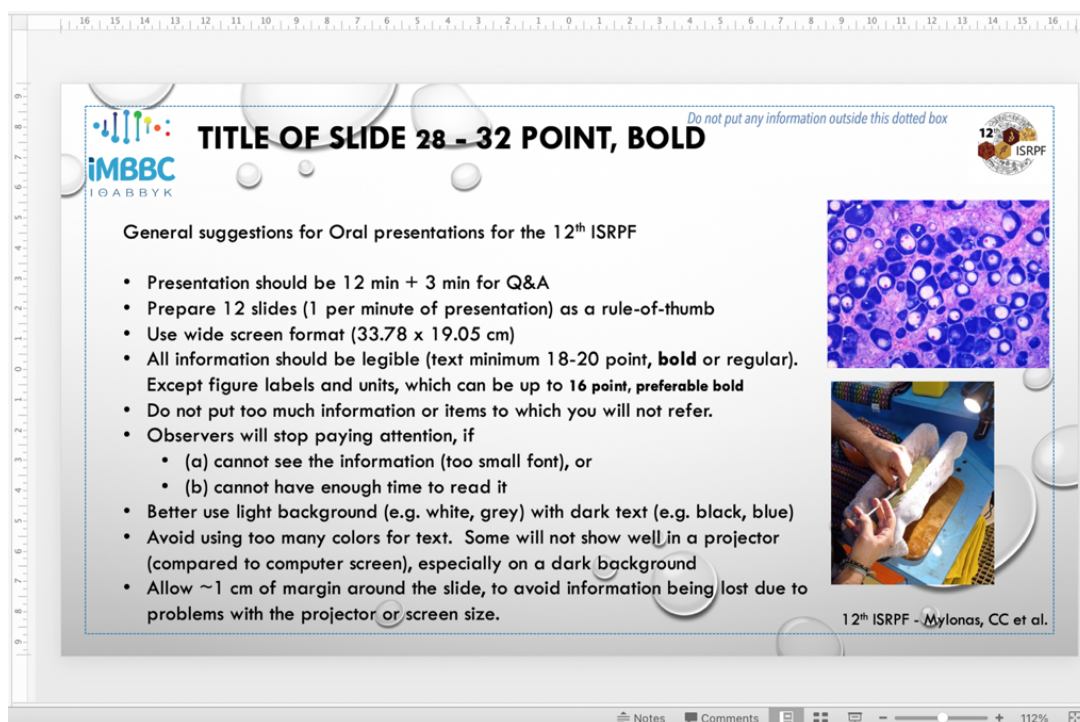
Poster presentations

The accepted size for the Posters is A0 upright (119 cm height x 84 cm width). The poster must be printed on gloss or matt paper, or light fabric, but **not heavy canvas or rigid plasticize material**, which may be too heavy to stay on the board! We will provide pins, double sided tape, and blue tag for putting up the posters. Posters should be placed on their corresponding board on Sunday 14 or at the latest Monday 15 May 2023 in the morning, and will be organized according to Special Session. Each Poster will be given a sequence number, and the boards will be numbered accordingly. Presenters having an odd number of poster (P1, P3 etc.) should stand by and present their posters during the Tuesday 16 May 2023 Poster Session (I). Presenters having an even number of poster (P2, P4, etc.) should stand by and present their posters during the Thursday 18 May 2023 Poster Session (II). Any new Abstracts submitted late (after 10 April 2023), will be given a sequence number from P101 onwards, and will be displayed in a different location than the Special Session to which they are allocated, at the end of Special Session 9.

See later, for suggestions/advice on preparing your poster presentation.

General suggestions for Oral presentations for the 12th ISRPF

- Presentation should be **12 min + 3 min** for Q&A
- Prepare 12 slides (1 per minute of presentation) as a rule-of-thumb
- For the **Title of slide use 28 or 32-point text, bold**
- Use wide screen format (33.78 x 19.05 cm)
- All information should be legible (**text minimum 18-20 point, bold** or regular). Figure labels and units, may be up to **16 point, preferably bold**
- Do not put too much information or items to which you will not refer.
- Observers will stop paying attention, if
 - (a) they cannot see the information (too small font), or
 - (b) cannot have enough time to read it
- Better use light background (e.g. white, grey) with dark text (e.g. black, blue)
- Avoid using too many colors for text. Some colours will not show well in a projector (compared to computer screen), especially on a dark background
- Allow ~1 cm of margin around the slide, to avoid information being lost due to problems with the projector or screen size. **See sample slide below:**



Best Student Oral and Poster presentation

There will be two (2) awards for best **Student Oral Presentation** and two (2) awards for best **Student Poster Presentation**. The awards will also include a financial reward. The evaluation of the presentations will be made by members of the Scientific Program Committee, and the announcement and award ceremony will be held on Friday 19 May 2023, during the closing session (18:00). To be easier for the evaluation committee members to identify student posters and oral presentations, these have been identified in the Program and Book of Abstracts (student). It is suggested that an identification symbol is added to the poster itself (next to the photo of the presenter), as well as to the opening slide of the oral presentation (next to the title of the presentation), such as the **one provided here** (graduation cap and diploma).



General suggestions for Poster presentations for the 12th ISRPF

The accepted size for the Posters is A0 (119 cm height x 84 cm width) upright. The poster must be printed on gloss or matt paper, or light fabric, but not heavy fabric or rigid plasticize material, which may be too heavy to stay on the board!) Posters should be placed on their corresponding board on Sunday 14 or at the latest Monday 15 May 2023 in the morning, and will be organized according to Special Session. Each Poster will be given a sequence number, and the boards will be numbered accordingly. We will provide pins, double sided tape, and blue tag for anchoring the posters.

- Place a photo of the person **presenting the poster** next to the title. This will help participants find the presenter if they want to discuss about the poster.
- For the **Title of the poster use >60-point text, bold**
- All information should be legible (text minimum 36 point, **bold** or regular). Except figure labels and units, which can be up to **28 point, preferably bold**
- Observers will often not stand at a Poster, if
 - (a) they cannot read the information easily (too small font), or
 - (b) there is too much information and too many details
- Use eye-catching photos and graphics
- Focus more on the Objectives and less on the Methods
- Results are often easier to be read, if in a bullet format
- Conclusions should be clear and well-supported by the data and statistical analysis
- Display the logos of the affiliated University/Research Organization, as well as the project's Funding Agency
- Always acknowledge fully the Funding Agency and include the project's contract number

See sample poster below:

Evolution of sex ratio and egg production of gilthead seabream *Sparus aurata* over the course of five reproductive seasons

Dimitrios Karamanlidis^{1,2}, Eirini Sigelaki¹, Maria Papadaki¹, Ioannis Fakriadis^{1,2} and Constantinos C. Mylonas¹

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Introduction
Gilthead seabream (*Sparus aurata*) is a protandrous hermaphrodite, maturing first as males at 2 years of age, and then males convert progressively to females after 3 years of age. The aim of the present study was to examine the rate of sex reversal and the effect on egg production and quality over 5 consecutive reproductive seasons.

Materials and methods
Fish were monitored from 2009 until 2016 in two 5000-l tanks (Figure 1). Temperature ranged between 18.2 and 20°C (borehole) while photoperiod was natural. Egg quality was assessed daily, estimating fecundity (eggs kg⁻¹ body weight) and fertilization (%). In 2015, when the sex ratio was severely skewed, younger (smaller body weight) males were added and some females were removed from the tanks.

Table 1. Mean (±S.E.M.) reproductive parameters of gilthead seabream broodstocks (n=2) during 5 consecutive spawning seasons after first sexual maturity. Statistically significant differences are indicated by different letter superscripts. Non-significant differences are indicated by "ns".

Mean (±S.E.M.)	2012	2013	2014	2015	2016	P value
Sex ratio (males:females)	0.55 ±0.08 ^a	0.18±0.04 ^a	0.22±0.01 ^a	1.07±0.07 ^b	0.23±0.06 ^a	0.05
Daily relative fecundity (×10 ⁶ eggs kg ⁻¹)	15.1±3.1	17.5±1.7	12.5±1.0	9.9±1.7	10.3±2.5	ns
Total annual fecundity (×10 ⁶ eggs kg ⁻¹)	2587±205	3100±202	2239±7	1683±216	1694±679	ns
Fertilization (%)	83.5±9 ^a	92.1±1 ^a	92.1±1 ^a	82.8±9 ^a	66±10 ^b	0.05
Number of spawning days	141±3	180±19	191±19	156±6	131±4	ns
Male weight (kg)	1.15±0.02	1.20±0.03	1.32±0.02	1.19±0.07	1.28±0.14	ns
Female weight (kg)	1.27±0.01	1.49±0.05	1.71±0.06	1.90±0.12	1.77±0.04	ns

Results

- At the 2nd reproductive season (2012) 64% of the males from the 1st reproductive year had already converted to females (Table 1).
- At the 3rd reproductive year even more males converted to females, but thereafter the sex ratio stabilized in the stocks, being ~0.2 (i.e. 1:5 M:F).
- A year after the ratio was brought back to 1:1 by the addition of males and removal of females, the male:female ratio again became heavily skewed toward females, being again ~0.2 (i.e. 1:5 M:F, Table 1).
- The spawning period began in December and ended from mid-June to late July in all years studied, with high fecundity and fertilization success, except in the last year (Table 1).

Conclusions

- Gilthead seabream broodstock tend to stabilize their sex ratio at ~0.2 (i.e. 1:5 M:F) after the 3rd reproductive year (4 years old), even after addition of younger males and an attempt to correct the sex ratio to 1.
- No changes in egg production or quality were observed over the monitoring period of 5 reproductive seasons, with the exception of a reduction in fertilization success during the last year.

Sex differentiation and hermaphroditism of sharpnose seabream *Diplodus puntazzo* in captivity

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²Department of Science della Vita e dell'Ambiente, Università Politecnica delle Marche Via Bressa Bozco, 60131, Ancona, Italy.

Introduction
Sex differentiation in fishes is a labile process, depending on genetic and environmental factors, and hermaphroditism is very common. The aim of the present study was to acquire information on sex differentiation and sex strategy of sharpnose seabream (*Diplodus puntazzo*) in captivity during the first years of life. Sex identification is necessary for managing captive broodstocks in commercial aquaculture facilities and ensuring optimal egg production.

Materials and methods
A population of fish was studied for 2 years in order to describe sex differentiation and puberty. Growth and gonadal differentiation and development were examined every 30 days. Another population was studied for 3 years in order to monitor the sex strategy and hermaphroditism during consecutive reproductive seasons. Growth and gonadal development were examined in this population (n = 20-30, duplicate tanks) during the reproductive season at ages 1st, 2nd and 3rd. The reproductive status of the gonads was examined through histological processing.

SEX DIFFERENTIATION

Figure 1 shows histological images of the gonads at different stages: 0th year class, 1st spawning season, 2nd spawning season, and 3rd spawning season. The images show the transition from male (M) to female (F) and the presence of hermaphroditic individuals (MF).

RESULTS

- Gonadal differentiation commenced around 202 (dph) with the formation of the ovarian cavity (oc) (Photo A).
- Male differentiation commenced around 300 dph, as a histological gonad (Photo B). The ovarian cavity and primary oocytes (PO-never insert) could be noticed in the female part of the gonad, and spermatocytes (sc) in the male part (upper insert).
- Until reaching a total length (TL) of 170-210 mm achieved in 1st fish, all juveniles possessed bisexual gonads (Fig. 1). Bisexual fish were classified as "mf" with gonads consisting of equal amounts of testicular and ovarian tissue (Photo J). "mf" with predominantly testicular tissue with spermatocytes (sc) and oocytes (oc) (Photo C), but still having an ovarian cavity with primary oocytes (insert), "m" "mf" with predominantly ovarian tissue and a smaller testicular region (Photo D, insert).
- Until 2 years, fish were identified as either females (F) or bisexual. Males (M) were first observed at the 2nd year class (Photo E).
- Bisexual individuals were observed throughout the study, but in 3-year-old fish they were absent of the MF classification (Fig. 2).
- During the second reproductive period, females (F) and mf presented immature ovaries (Photo F) and males (M and MF) all types of germ cells, including sperm (Photo G). Surprisingly, all 3 "mf" fish found in this population presented more advanced oocyte development and more sperm compared to F, Dp, MF and M fish, with the ovarian tissue of mf fish presenting oocyte nuclei and the testicular tissue full of spermatozoa (Photo H).
- In the reproductive period of 3-year-old fish all females were classified as F and presented ovaries with oocytes and Vg oocytes (Photo K). All males (M and MF) were oocytes (Photo I).

Figure 1. The first males (M) and females (F) exhibiting the tissue of the opposite sex, appeared in fish longer than 210 mm. M = functional male, MF = predominantly testicular tissue in gonad, mf = equal testicular and ovarian regions in gonad, mf = predominantly ovarian tissue in gonad, F = functional female.

Figure 2. Sex ratio of functional males (M) and MF class, functional females (mf) and F class were 1:1, 2:0 and 2:1 for 1st, 2nd and 3rd year class, respectively. Legend: mf = 1, F = 1.

Discussion

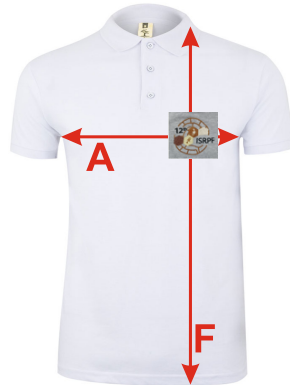
- Data support the existence of rudimentary hermaphroditism in sharpnose seabream.
- Bisexual MF individuals develop into males, mf into females and mf are possibly the ones changing sex by the degeneration of the testicular and the development of the ovarian tissue.
- The presence of individuals exhibiting bisexual gonads with repressed testicular tissue indicates the occurrence of partial protandry in the first years of life.
- The study will continue until fish are 5th year old, but so far it seems that the sex ratio of cultured sharpnose seabream broodstock may stabilize after their third year of life.

Financial support for U.S. has been provided by the project "Campus World per stage all'estero" of the Università Politecnica delle Marche (Ancona, Italy).

T-shirts

We have produced a Polo t-shirt with the logo of the symposium. The t-shirts will be available at the registration desk, beginning on Tuesday 16 May 2023. There is only one color and a unisex-style, in sizes S, M, L, XL, 2XL, 3XL and 4XL, according to the chart below (in cm).

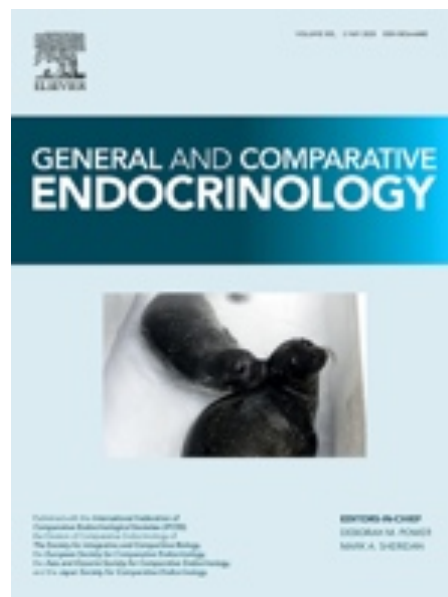
MK215	1/2 Pecho 1/2 Chest	Largo delantero Front length
SIZE	A	F
S	50	69
M	53	71
L	56	73
XL	59	75
2XL	63	77
3XL	66	80
4XL	69	83
5XL	72	86



Special Issue in the journal General and Comparative Endocrinology

As for previous ISRPFs, we will have a Special Issue (SI) in the journal General and Comparative Endocrinology (GCE) with submitted manuscripts from the 12th ISRPFs, from both Oral and Poster presentations. The SI will be edited by Mylonas, CC., Bobe, J., Piferrer, F. and Schulz, R., who will coordinate the review process, according to the specific area of the submitted manuscripts.

Submission of the manuscript should be done through the regular platform of GCE, **beginning on 1 June 2023, and the deadline for submitting a manuscript will be 31 August 2023**, so that the review can be completed by the end of the year, and the SI published in the beginning of 2024. As before, manuscripts considered for publication should not necessarily include “endocrine” work or data, since the ISRPF includes research in all aspects of Fish Reproductive Physiology and not just endocrinology. However, they must come from work presented in the 12th ISRPF or by an author registered in the symposium presenting other work in the area of Fish Reproductive Physiology.



Program at a glance

Sunday 14 May	Monday 15 May	Tuesday 16 May	Wednesday 17 May	Thursday 18 May	Friday 19 May
14:30 – 18:00 Registration	08:30 – 09:00 Welcoming Dr. Constantinos (Dinos) C. Mylonas, HCMR, Greece	09:00 – 10:00 Plenary by Pr. Daniel Pauly, UBC, Canada	09:00 – 12:15 SS5. Climate change & anthropogenic impacts (10:30 -11:00 Coffee break)	09:00 – 12:00 SS6. Reproduction in aquaculture (10:30 -11:00 Coffee break)	10:00 – 13:00 SS8. Behaviour & pheromones (11:30 -12:00 Coffee break)
19:30 Welcome reception (dinner and drinks)	09:00 – 10:00 Plenary by Dr. Sylvie Dufour, CNRS, France	10:00 – 13:00 SS3. Oogenesis/ vitellogenesis & ovulation (11:00 – 11:30 Coffee break)	12:15 – 13:30 Lunch	12:00 – 12:15 Group Photo	13:00 – 14:30 Lunch
	10:00 – 13:00 SS1. Sex determination & Differentiation (11:00 – 11:30 Coffee break)	13:00 – 14:30 Lunch	13:30 – 19:30 *Excursion to Knossos Palace (Heraklion) or Spinalonga Island (Lasithi)	12:15 – 13:30 Lunch	14:30 – 17:30 SS9. Reproductive biotechnologies (16:00 -16:30 Coffee break)
	13:00 – 14:30 Lunch	14:30-17:00 SS4. Spermatogenesis & spermiation		13:30 – 16:00 SS7. Gamete & egg quality	17:30 – 18:00 Summary Birgitta Norberg, IMR, Norway
	14:30-17:30 SS2. Brain- pituitary – gonad axis (16:00- 16:30 Coffee Break)	17:00- 19:00 Poster 1 (Odd numbers) & Coffee break		16:00 -18:00 Poster 2 (Even numbers) & Coffee break	18:00 – 18:30 Closing Dr. Constantinos (Dinos) C. Mylonas, HCMR, Greece
				19:00 *Symposium Dinner	

*Optional and at additional charge but **free of charge** for students with valid University ID.

Session chairs, co-chairs and State-of-the-art speakers

Special Session	Chair	Co-chair	State-of-the-art speaker
1. Sex determination and differentiation	Piferrer, Francesc	Chang, Ching-Fong	Shao, Changwei
2. Brain-pituitary-gonad axis	Levavi-Sivan, Berta	Golan, Matan	Golan, Matan
3. Oogenesis/vitellogenesis and ovulation	Rosenfeld, Hanna	Yilmaz, Ozlem	Yilmaz, Ozlem
4. Spermatogenesis and spermiation	Schulz, Rüdiger	Chauvigné, François	Crespo, Diego
5. Climate change and anthropogenic impacts	Norberg, Birgitta	Carnevali, Oliana	Servili, Ariana Moreira, Renata
6. Reproduction in aquaculture	Migaud, Hervé	Horvath, Akos	Akos Horvath
7. Gamete and egg quality	Bobé, Julién	Żarski, Daniel	Żarski, Daniel
8. Behaviour and pheromones	Duncan, Neil	Li, Weiming	Li, Weiming
9. Reproductive biotechnologies	Zohar, Yonathan	Yoshizaki, Goro	Yoshizaki, Goro

Social activities

Welcome reception – Sunday 14 May 2023 (19:00)

The ISRPF 2023 Welcome Reception will take place at the **Aldemar Knossos Royal Beach Resort**, which offers the ideal scenery for welcoming the participants to Crete and to see old friends and colleagues, and meet new students and young researchers! The Welcome Reception is **free for all participants**, courtesy of the Hellenic Aquaculture Producers Organization. We will get to sample fish from the Greek aquaculture industry, courtesy of Galaxidi Marine Farm SA.



Group Excursion - Wednesday 17 May 2023 after lunch (13:30)

Wednesday afternoon is devoted to socializing and networking. Participants may visit some of the most important historical sites of Crete, at an extra cost for regular registrants (45-47€), but **free for students**, courtesy of IRIDA SA. There are two options available: the Knossos Palace (Heraklion Region) or Spinalonga Island (Lassithi Region).



Guided tour to the Knossos Palace (the cradle of the Minoan Civilization)

Amongst all the ancient monuments in Crete, Knossos is the archaeological site that is a must-see in order to understand the greatness of the Minoan Civilization (2000 BC). The tour takes you to the island's capital, Iraklio (Heraklion), and from there onto the village of Knossos where you will be led through the ruins of the ancient palace complex: the labyrinth, galleries and rooms of the Palace, and the Royal Palace of King Minos, the son Zeus (the God of Gods, in Greek Mythology) and Europa, who gave her name to our continent! Return to the hotel is after approximately 5 hours. The excursion includes transfers to/from Knossos Palace with luxury a/c coaches, a professional-official English-speaking guide per coach, and entrance fee and full guidance in Knossos Palace.



Guided tour to Spinalonga Island (a Venetian fortress, an Ottoman village and a Leper colony)



Participants will be picked up from the hotel and transferred to the town of Elounda and then with a 10 min boat ride across to the island of Spinalonga, which has an exceptionally interesting history. Once a Venetian Fortress built in 1579 and later an Ottoman village, in the 20th century it has become synonymous with human pain. In 1903, Spinalonga developed into a gathering place for people infected with Leprosy (Hansen's disease) from all over Crete, where they spent the rest of their life! Today, with the painful story forgotten, the colony is being restored and it offers a pleasant visit with spectacular scenery! After this nice visit the boat will bring participants back at Elounda or Ag. Nikolaos. The excursion includes transfers to/from Elounda with luxury a/c coach, a professional-official English-speaking guide per coach, boat ride to/from Spinalonga Island, and entrance fee and full guidance to the Island.

Symposium dinner – Thursday 18 May 2023, evening (19:00)

Due to the fact that the weather is not stable yet and the evenings are quite cool, the Symposium dinner will take place at a celebrations and event hall (Garden of the Senses, https://www.facebook.com/kiposaisthiseon/?locale=el_GR). The transfers between the venue and the hall will be done by coach, leaving the entrance of the venue ([Aldemar Knossos Royal Beach Resort](#)) at 19:00. One bus will depart from the hall to bring participants back to the hotel at 23:00. The remaining buses will depart from the restaurant between 24:00-01:00, depending on how the party goes!

The Symposium dinner is optional and at an extra cost for regular registrants (50 € euro), but **free for students** (courtesy of IRIDA SA). We are sure that all will enjoy the hospitality and festive atmosphere in a local “γλέντι” (translated as “party”, pronounced “glendi”) with Cretan food and folk dances, so get your ticket too!

Travel information

The venue of the 12 ISRPF will be the [Aldemar Knossos Royal Beach Resort](#), located in the area of Chersonissos, Crete, Greece.

Since Crete is an island, you can arrive by airplane from Athens or directly from several European cities. The national airlines are **Aegean Airways and Olympic Airways** (affiliated with **Star Alliance**). Crete is also accessible daily by ferry from Athens (Piraeus Harbor) and the trip takes 9 hrs (21:30 - 06:30). The venue is 25 km from the **international airport of Heraklion** (also spelled Iraklio), “**Nikos Kazantzakis**” (**HER**).

From the airport in Heraklion, you can get to the venue in Chersonissos by **bus** or **taxi**. You can also rent a car, from a large number of different companies (both International and local). **Be careful if you will drive yourself**. Greeks are notorious of being “adventurous” drivers, and the road system in Crete is not the best!

The **bus station** is located at the entrance of the airport site, at the right side of the traffic lights as you are looking away from the airport. The bus will get you from Heraklion Airport to Chersonissos in about 45 minutes, and the ticket will cost 3-5€. Tickets can be bought from the bus driver when boarding or in a ticket kiosk at the bus stop.

Taxis are available right at the exit of the airport building (arrivals). A taxi trip will last about 25 minutes, and its cost is 40-50€.

So, pack your bags, get your presentation ready and/or poster printed, and come to Crete for one more version of the ISRPF! We are waiting to greet you and spent an unforgettable time together!

See you soon!



Constantinos (Dinos) C Mylonas

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Rüdiger Schulz, Uni Utrecht, Netherlands
Yonathan Zohar, IMET-Uni Maryland, USA

Fish cultured in Greece and the Mediterranean Sea

Dicentrarchus labrax



Sparus aurata



Argyrosomus regius



Pagrus major

Seriola dumerili



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