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## **A Histological Examination of the Ovaries of Pacific Sanddab, *Citharichthys sordidus*, Captured at Two Oil Platforms and Two Natural Sites in the Southern California Bight**

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*Abstract.*—A number of the 26 offshore oil and gas platforms off California may be nearing the end of their economic lives. Decisions as to the disposition of these platforms will be based on a number of parameters, including the biological role of the structures. One issue that has arisen is the possible contamination of fishes living around platforms resulting from contaminants released during drilling and production. If significant contamination is occurring, it would be expected to impair the reproductive abilities of impacted fishes. One form of reproductive impairment is atresia, the abnormal reabsorption of oocytes that are destined to be spawned. Atresia has been widely used as an indicator of pollutant-related reproductive impairment in fishes. We examined the occurrence of atretic oocytes in Pacific sanddab, *Citharichthys sordidus*, collected near two offshore platforms in the Santa Barbara Channel (B and Gilda) and from two natural reference sites (off the east end of Santa Cruz Island and in mid-channel off Rincon). While pronounced atresia was observed in a few fish at one natural site and one platform, there was no evidence of widespread pronounced atresia at any of the four sites.

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There are 26 offshore oil and gas platforms in California waters, many of which may be nearing the ends of their economic life spans. Following the decision by an operator to cease production, the process by which a decision is made as to the disposition of a platform is called decommissioning. This procedure may take into consideration the biological, sociological, and economic consequences of various decommissioning options, which range from leaving the entire structure in place through partial or total removal (Schroeder and Love 2004).

Among the issues that need to be addressed in this process is the possible contamination of platform-dwelling organisms. A number of activities associated with the production of oil and gas result in the creation of contaminants. Drilling muds (used to lubricate drill bits), drill cuttings (the hard material brought up by the drill bit), the water associated with the petroleum produced (“produced water”), and water used as coolant contain a variety of elements and compounds, including petroleum hydrocarbons (such as polycyclic aromatic hydrocarbons (PAHs)) and metals (Utvik 1999; Neff 2002; Steinberger et al. 2004). At least some of these elements and compounds are released into the water and may be deposited on the sea floor around these structures (Grant and

Briggs 2002; Phillips et al. 2006). While the acute toxicity of these materials varies considerably among both platforms and organisms (Utvik 1999; Grant and Briggs 2002), less is known about their potential chronic effects (Stephens et al. 2000).

One issue to be addressed during decommissioning is the possible damage to fish reproduction, by pollutants, if a structure is left in place. Pollutants such as heavy metals, PAHs, and chlorinated hydrocarbons have been linked to a range of physiological problems in fishes, including reproductive impairment (Hose et al. 1989; Cross and Hose 1988; Thomas 1989). One form of reproductive impairment linked to pollution is atresia, the abnormal reabsorption of oocytes destined to be spawned. Atresia has been used as an indicator of pollutant-related reproductive impairment in fish living in the southern California Bight (Cross et al. 1984).

The objective of this investigation was to compare the spawning capability, as measured by pronounced atresia, of Pacific sanddab (*Citharichthys sordidus*) living around two oil platforms with fish inhabiting two natural areas. This species occurs from the western Gulf of Alaska to Bahia Magdalena, southern Baja California, and as an isolated population in the Gulf of California (Love et al. 2005). Pacific sanddab are very widespread throughout southern California waters and live both on soft sea floors at natural sites and around oil and gas platforms (Love et al. 1999; Allen et al. 2007). This flatfish spends much of its time lying on or partially covered in sediment, although we have also seen them well up in the water column. It is an opportunistic feeder, preying on such benthic infauna as polychaetes, as well as such benthic, epibenthic, and water column organisms including shrimps, gammarid amphipods, mysids, euphausiids, squids, and fishes (Allen 1982; Allen et al. 2002). Pacific sanddab spawn from late spring through late fall, probably peaking in the summer (Arora 1951; M. McCrea, pers. comm.). The pattern of chlorinated hydrocarbon contamination of Pacific sanddab in the Southern California Bight, which demonstrates high levels only around the Palos Verdes Whites Point Outfall (a significant source of contamination), implies that these fish do not make large-scale movements (Schiff and Allen 2000).

Based on its life history, it would be expected that the Pacific sanddab is an excellent species on which to model the reproductive effects of platform discharges. This fish is likely to come into contact with benthic pollutants, both through direct physical contact and absorption, and from its diet of both sea-floor infauna and epibenthic organisms. In fact, Pacific sanddab have been shown to bioaccumulate pollutants and have been used as tools to assess levels of tissue contamination (Schiff and Allen 2000; Allen et al. 2002) and to determine sources of pollution (Parnell et al. 2008).

### Methods

Pacific sanddab were collected at four sites in the Santa Barbara Channel, southern California, by hook and line in September 2005 (Fig. 1, Table 1). The sampling sites were 1) Platform B, 2) Platform Gilda 3) a natural site located about 8 km offshore and southeast of Platform B ("Rincon"), and 4) a natural site off the northeast corner of Santa Cruz Island ("Santa Cruz Island"). Both platforms have produced oil and gas for many years, Platform B starting in 1968 and Platform Gilda in 1981 (Love et al. 2003). Platform fish were collected within 30–60 m of each structure.

After capture, fish were placed on ice and several hours later were measured (total length, cm), the ovaries fixed in 10% formalin, and later shipped to Dr. Stephen R. Goldberg. A section of each ovary was removed, dehydrated in ethanol of ascending concentrations, embedded in paraffin and serial sections were cut at 5  $\mu$ m using a rotary

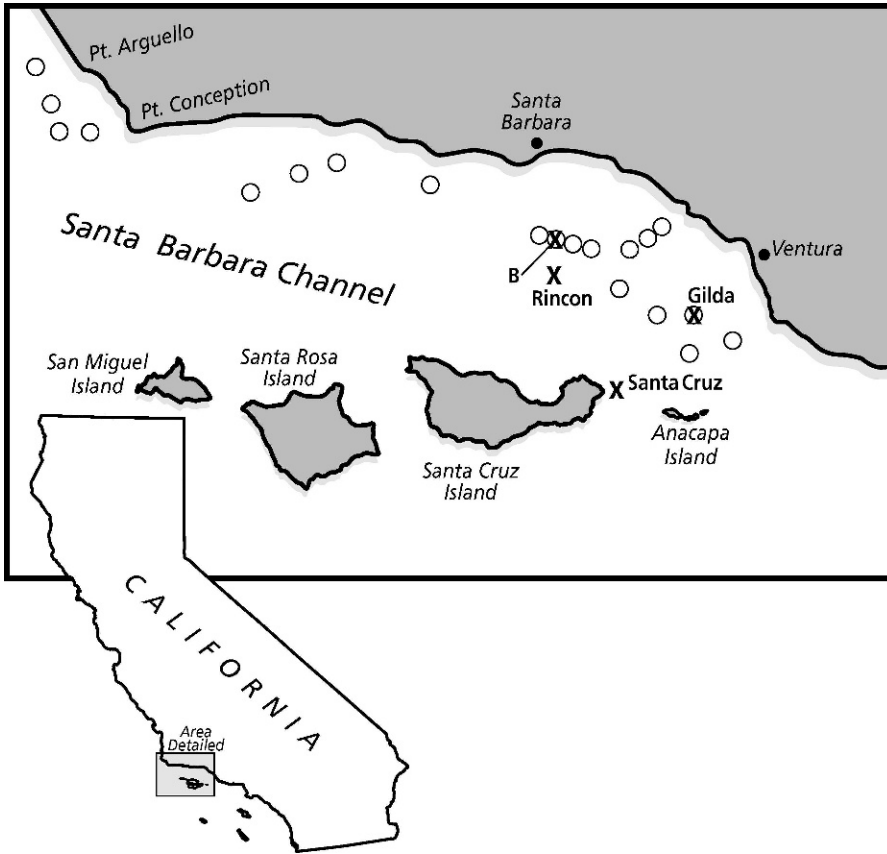


Fig. 1. The location of the four Pacific sanddab sampling sites (latitudes and longitudes listed in Table 1). Circles represent all of the platforms off California and those with an “x” inside are the two study platforms.

microtome. Sections were mounted on glass slides, stained with hematoxylin followed by eosin counterstain. Slides from each ovary were examined under a compound microscope. The presence of the following was noted: (1) hydrated eggs for upcoming spawning; (2) vitellogenesis (yolk deposition) in mode of smaller eggs for subsequent spawning; (3) postovulatory follicles (evidence of recent spawning); (4) follicular atresia (degenerating oocytes). Atresia was characterized as minor (<10% of eggs atretic) or pronounced ( $\geq 10\%$  of eggs atretic) (Cross et al. 1984; McDermott et al. 2007).

Table 1. Details of Pacific sanddab, *Citharichthys sordidus*, collections made between 11 and 20 September 2005 in the Santa Barbara Channel.

Site	Date	Depth (m)	Location	n	TL (cm) $\pm$ SD
Platform B	11 Sept.	61 m	34°19'56" 119°37'18"	18	16.1 $\pm$ 1.6
Rincon	16 Sept.	87 m	34°15'21" 119°33'14"	19	19.3 $\pm$ 4.8
Platform Gilda	20 Sept.	64 m	34°10'56" 119°25'09"	20	20.8 $\pm$ 2.6
Santa Cruz I.	20 Sept.	64 m	34°01'48" 119°30'22"	21	20.7 $\pm$ 3.4

## Results

Within Pacific sanddab ovaries, we observed (1) hydrated eggs, (2) smaller eggs with yolks that would later grow and hydrate prior to spawning, (3) postovulatory follicles, (4) minor atresia, and (5) pronounced atresia (Figs. 2A–C, Table 2).

Egg hydration (Fig. 2A) occurs just prior to spawning, when a mature oocyte expands to as much as four times its original volume (Wallace and Selman 1981). Between 100% (Platform Gilda) and 50% (Rincon) of fish at each site contained hydrated eggs (Table 2). Smaller yolked eggs were found in between 100% (Platform Gilda) and 55% (Rincon) of fish (Table 2). Postovulatory follicles (Fig. 2B) are remnants of the granulosa layer of the previously spawned egg that hypertrophies after the oocyte is released and thus their presence demonstrates that a female has spawned earlier in the season. Older postovulatory follicles (> 24 hours) can be distinguished from “fresh” ones by deterioration of original shape and loss of organization. The presence of postovulatory follicles from a recent spawning alongside maturing follicles destined for a subsequent spawning (Fig. 2B) indicates Pacific sanddab spawn more than once during a reproductive season. Postovulatory follicles were found in between 65% (Santa Cruz Island) and 5% (Rincon) of fish (Table 2). At least some fish with minor atresia (Fig. 2C) were noted at all of the sites, with levels ranging from 60% (Platform Gilda) to 15% at Santa Cruz Island. Pronounced atresia was relatively rare. No fish at Platform Gilda and Santa Cruz Island were identified as having pronounced atresia, while 6% of the sanddabs at Platform B and 16% at Rincon exhibited this condition (Table 2).

## Discussion

In general, we observed relatively little evidence of conspicuous reproductive impairment in Pacific sanddab from both platforms and natural sites. Overall, most fish contained hydrated oocytes and were about to spawn and most contained oocytes with smaller yolks and thus were likely to spawn again. Many fish exhibited minor atresia. Atresia is the spontaneous degeneration of an oocyte at any stage in its development and it occurs at low frequency throughout the ovarian cycle. For instance, background (i.e., non-test) levels of minor atresia in laboratory-raised zebrafish, *Danio rerio*, were 58% (Rossteuscher et al. 2008). The frequency of minor atresia typically increases toward the end of the spawning cycle when follicles that initiated, but did not complete, yolk deposition degenerate (Goldberg, 1981). The lack of any evidence of substantial reproductive impairment in fish living around two oil and gas platforms implies that large-scale reproductive damage is unlikely to be occurring.

We observed relatively few instances of pronounced atresia, a condition that is only occasionally observed in unstressed populations. As an example, pronounced atresia was only found in 2% of female Atka mackerel, *Pleurogrammus monopterygius*, taken from the relatively pristine Aleutian Islands (McDermott et al. 2007). A wide range of environmental stressors, including heavy metals (Pierron et al. 2008), endocrine disrupters (Pollino et al. 2007), and starvation and lipid-poor diets (Hunter and Macewicz 1985; Sherwood et al. 2007) can cause pronounced atresia. The level of pronounced atresia we documented was much less than that found in a study of longspine combfish, *Zaniolepis latipinnis*, and yellowchin sculpin, *Icelinus quadriseriatus*, from the vicinity of sewage outfalls in Santa Monica Bay and off Palos Verdes, southern California, or from a control site in Santa Monica Bay (Cross et al. 1984). That study documented high levels of pronounced atresia in 28–49% of females from around sewage outfalls and 42–44% from a control site (that was apparently also heavily polluted).

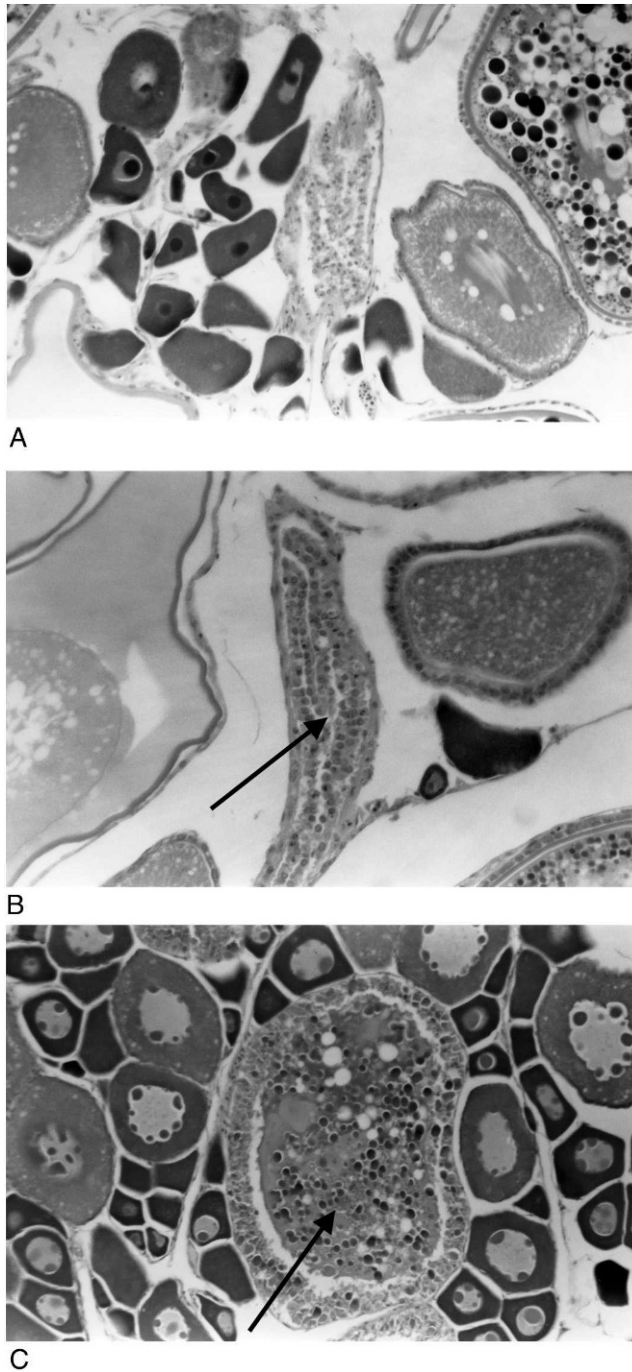


Fig. 2. A) Ovary of Pacific sanddab showing older postovulatory follicle (center); B) ovary of Pacific sanddab showing hydrated oocyte (left), yolk deposition in follicle (lower right), and fresh postovulatory follicle in center; C) ovary of Pacific sanddab showing atretic follicle (center). Note enlarged height of granulosa cell layer and ingested yolk granules. All images at 160  $\times$ .

Table 2. Histological analyses of Pacific sanddab ovaries from four sites in the Santa Barbara Channel. Values in each column are in percentages of individuals sampled.

Site	n	Hydrated eggs	Yolk in smaller modes	Post-ovulatory follicles	Minor atresia	Pronounced atresia
B	18	95	95	61	22	6
Rincon	19	50	55	5	35	16
Gilda	20	100	100	35	60	0
SCI	21	85	85	65	15	0

It might be argued that there was some reduction in the reproductive capacity of fish from Rincon as fewer fish from that site harbored 1) hydrated oocytes, 2) those smaller oocytes destined for later spawning, and 3) post-ovulatory oocytes. Furthermore, 16% of female Pacific sanddab from Rincon contained ovaries with pronounced atresia. We note that our sample size from Rincon was relatively small, encompassed only one month, and the factors responsible for the reduction in ovarian output in these females remain to be determined.

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