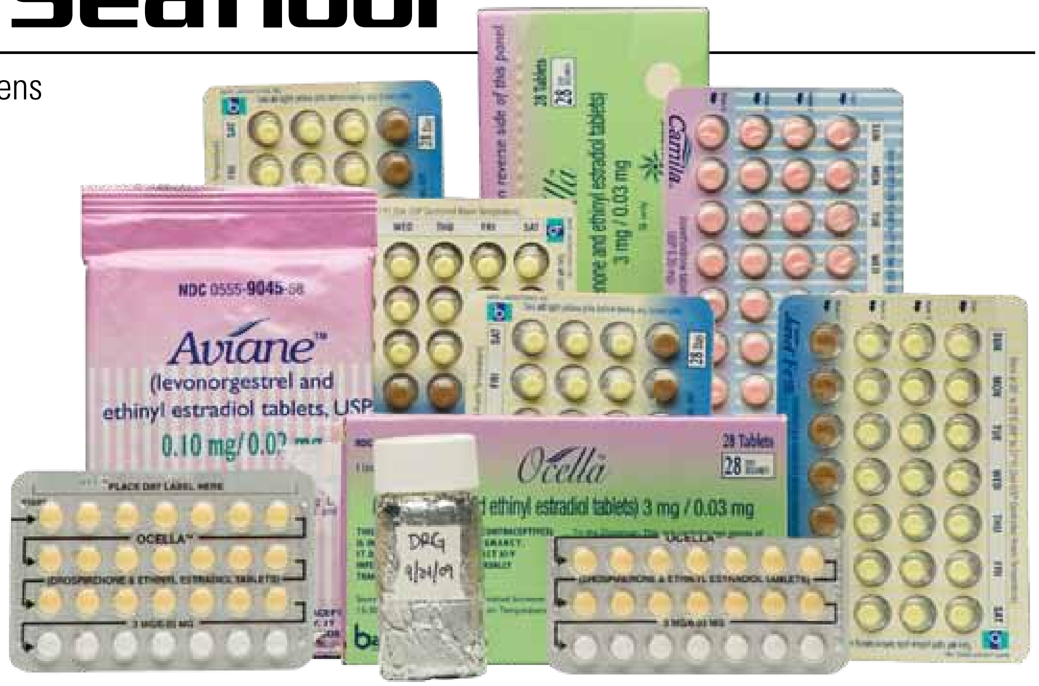
From Sewers to the Seafloor

What impacts might estrogens have in the coastal ocean?

DAVID GRIFFITH
MARINE CHEMISTRY & GEOCHEMISTRY



Tom Kleindinst, WHOI

Synthetic estrogens in birth control pills can enter the ocean after being excreted from the body and when pills are flushed down toilets.

On a crisp October morning, our small boat bobbed gently ten miles offshore. The sun glinted off the dark blue surface of Massachusetts Bay and directly below us, all of Boston's sewage was surging unseen into the ocean. Back on shore the city was waking up and flushing toilets, and the ensuing "morning rush" had just begun its nineteen-hour journey to this place. Out of sight, this plume would be easy to ignore if it didn't contain hard-to-detect chemicals that could have impacts on our health and the environment.

We had spent weeks preparing for our first sampling trip, and I crossed my fingers as we began setting up the equipment we would use to stalk an elusive chemical in the ocean.

If you are a human or any other animal with a backbone, your body makes estrogen. And every day, you send some portion down the toilet to your local sewage treatment plant or septic system. Treated or not, these waste streams ultimately flow into nearby groundwater, rivers, lakes, and coastal waters that often supply the water we drink and the fish we eat.

Estrogens are a family of hormones that are essential for growth and development, and, notably, for determining whether you are a female or a male. Exposure to even small amounts of "extra" estrogen can have profoundly negative health effects, giving rise to male fish with female sex organs, for example.

In 2001, to test just how potent estrogens could be, researchers at Fisheries and Oceans Canada and the United States Environmental Protection Agency began an experiment. They added a synthetic estrogen commonly found in birth control pills to a pristine lake in Ontario at levels typical of those detected in treated sewage. They found that within two years, the lake's entire population of fathead minnows had collapsed because they could no longer reproduce.

Some 360 million gallons of sewage is released per day from Boston's sewage treatment plant on Deer Island. What type of estrogens get into Massachusetts Bay?

Jack Cook, WHOI

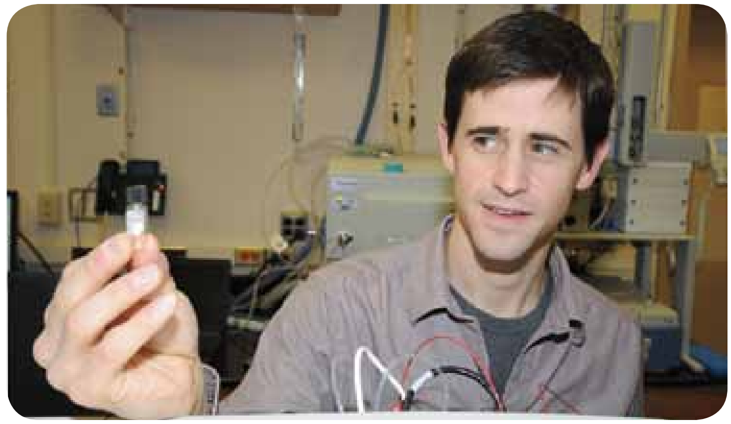
My own research aims to characterize the amounts, variety, and ultimate fate of estrogens discharged with sewage into Massachusetts Bay. Until now, scientists have primarily focused on a single form of estrogen. However, we are looking for a wide array, including forms that have escaped notice in the past, and we are looking for them at very low concentrations. Fortunately, recent advances in detector technology have given us the ability to pick out estrogens from the muddle of the millions of other compounds in seawater.

Is dilution the solution?

On an average day, greater Boston releases 360 million gallons of treated sewage into Massachusetts Bay through a 24-foot-wide pipe that could fit two semi-trailers side by side. This is a tiny fraction of the flow of treated sewage worldwide, but it is huge nonetheless—roughly equivalent to driving seven semi-trailers' full of treated sewage into the bay every minute of every day.

Below our boat that October day, perhaps only a couple teaspoons' worth of estrogens flowed into the water. Massachusetts Bay contains enough water to fill 50 million Olympic swimming pools and is continually flushed by water from the neighboring Atlantic Ocean. Mix those few teaspoons of estrogen into the bay and you have one extremely dilute solution of estrogen—but nevertheless, a solution well worth studying because estrogens are potent at minuscule (parts per trillion) concentrations. We still know very little about the kinds and amounts of estrogens that end up in our groundwater, rivers, and lakes. We know even less about estrogens in seawater.

For many years, Boston's treated sewage was released directly into the harbor, and contaminants and excessive nutrients in the sewage visibly impaired the health of this relatively small body of water. In 2000, after several years of research, planning, and construction, and at great expense, Boston's treated sewage was rerouted offshore through 9.5 miles of bedrock and a long series of 55 "diffusers" that empty onto the floor of Massachusetts Bay, under 100 feet of water. The treated effluent is more diluted here because it mixes with a much larger body of water.



In the lab at WHOI, David Griffith analyzes samples of seawater he collected in Massachusetts Bay to detect minute quantities of estrogens in the ocean.

Ken Kostel, WHOI

If dilution were the only process at work, then determining estrogen concentrations would be straightforward. They would depend on the total amount of estrogen discharged with sewage, the volume of Massachusetts Bay, and the average time a water parcel spends there.

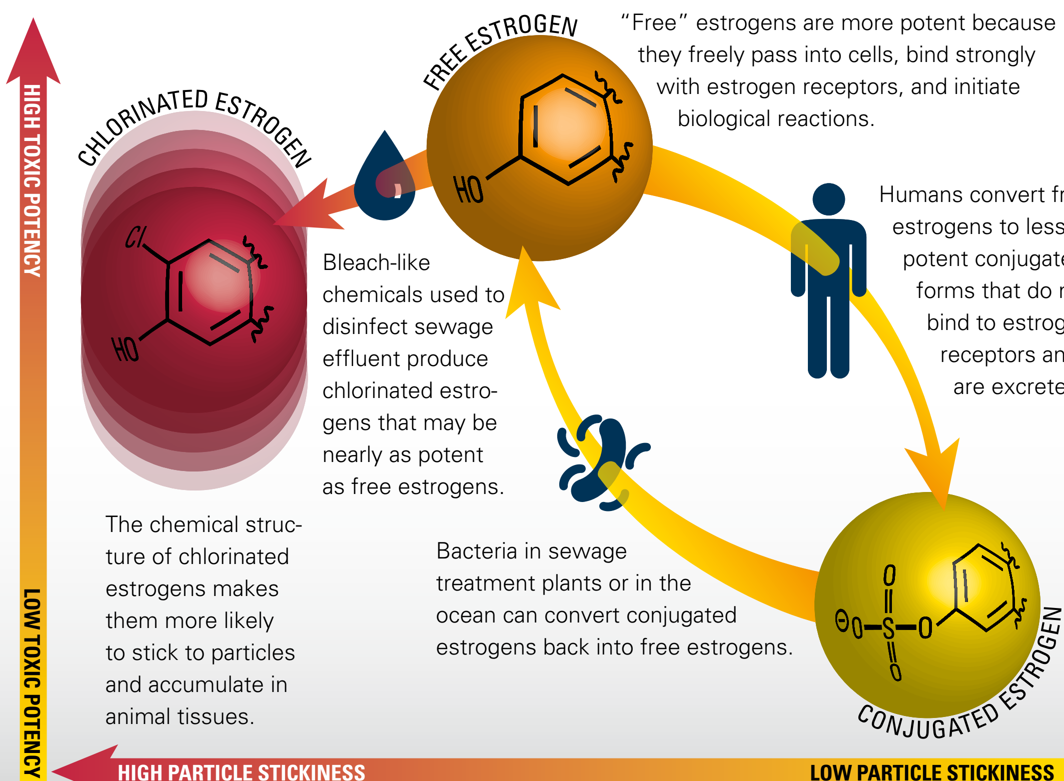
But some estrogens are removed from the water over time by a variety of natural processes. Estrogens can get swept away by currents. They may also stick to particles and sink to the seafloor, accumulate in animal tissues, be altered by bacteria, or be destroyed by sunlight in surface waters.

And it is likely that treated sewage isn't the only source of estrogen. When it rains, pulses of estrogens probably enter the coastal ocean as an overflow of storm water temporarily overwhelms sewage treatment plants, and they are forced to shunt untreated or partially treated sewage into the harbor.

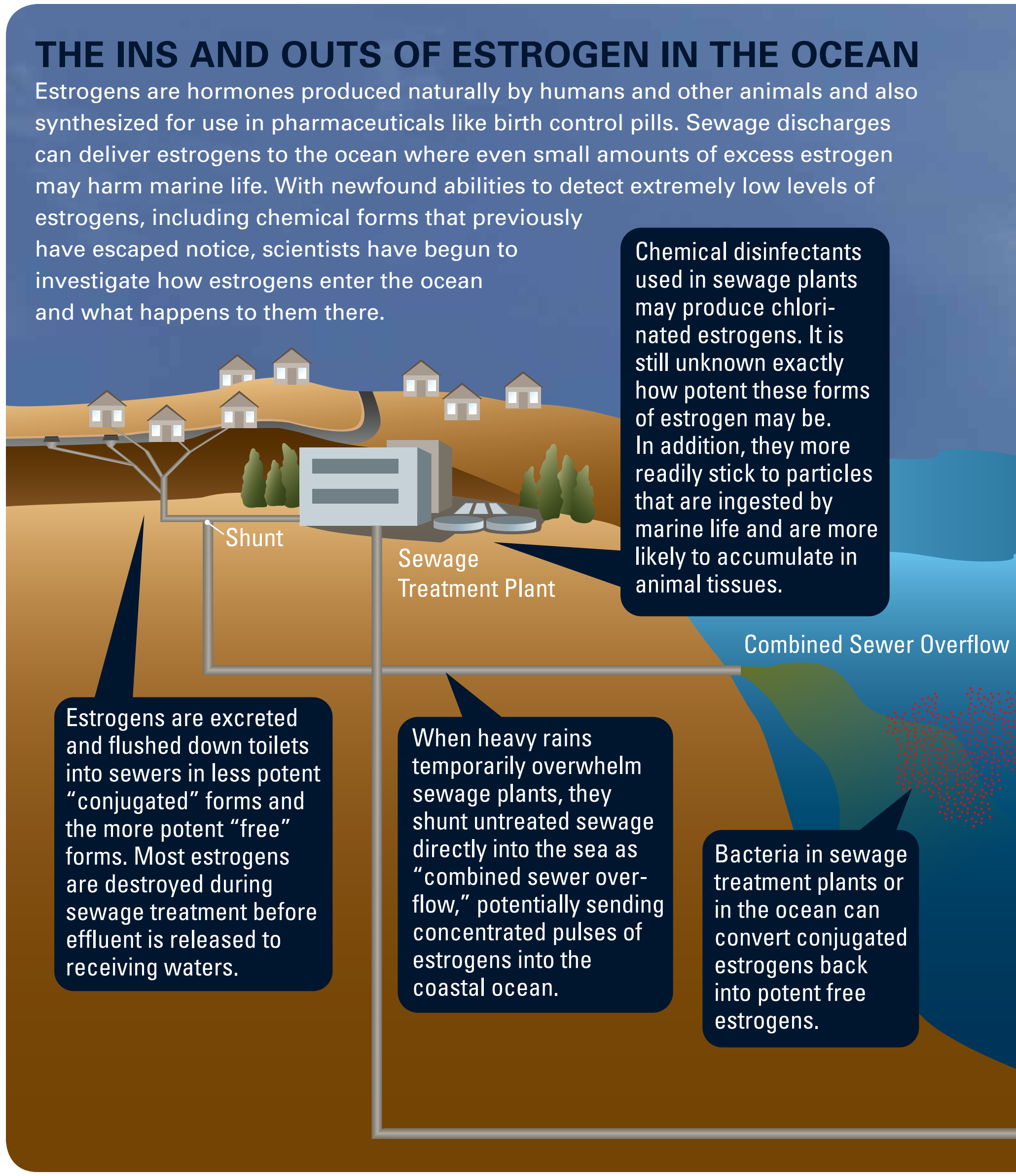
Which of these processes are most important? To find out, we first have to measure how much of each type of estrogen is in the bay.

ESTROGENS COME IN SEVERAL 'FLAVORS'

Estrogens are hormones that are essential for growth and development, but even minuscule amounts of excess estrogen can have detrimental health impacts on living things. The core of estrogens is composed of 18 carbon atoms bonded together into four rings. Slight chemical differences in the first ring produce significant changes in the estrogens' potency.



Amy Caracappa-Qubeck, WHOI



Amy Caracappa-Qubeck, WHOI

A wide variety of estrogens

There is a maxim in the field of toxicology: "The dose makes the poison." This is why eating the trace amounts of cyanide in an apple seed is safe, but drinking too much water can kill you. The maxim also holds for concentrations of estrogen in coastal waters. But distinguishing "harmful" from "safe" is a real challenge—particularly when the threshold lies near the lower limit of detection. This is common for potent substances like estrogens.

In my current research, with advisors Philip Gschwend at MIT and Timothy Eglinton at ETH-Zurich, we are developing methods to detect and identify estrogens. First we pump 10 to 20 gallons of seawater through thin porous disks about the size and thickness of a CD, made from a material that is "sticky" to estrogens and many other compounds in the water. We then return to the lab and soak our disks in a small amount of alcohol, which pulls estrogens off the disks and into the liquid. By evaporating most of the alcohol, we can concentrate our sample down to 1 milliliter (less than a ¼ teaspoon) before injecting 1 percent of that solution into an instrument called a liquid chromatograph mass spectrometer. The LC-MS, as it is called, can separate and detect different estrogens based on their specific structure and mass (see Page 19).

Estrogens take a variety of forms, the most potent of which are "free" estrogens, so called because they freely pass into cells and bind with estrogen receptors, initiating a cascade of biological responses. One particularly potent synthetic free estrogen, 17α-ethynylestradiol, has been widely used in birth control pills since the 1960s. Hans Inhoffen and his colleagues at Schering A.G. in Berlin first synthesized 17α-ethynylestradiol in 1938 by adding two extra carbon atoms to a standard form of estrogen. This slight difference in chemical structure makes 17α-ethynylestradiol more potent than natural estrogens, and more likely to persist in the environment.

Our bodies regularly produce a fresh supply of estrogens. To maintain a balance, certain enzymes convert our existing free estrogens into "conjugated" forms that can be excreted in feces and urine. (The conversion involves attaching a sulfate or glucuronide group to an oxygen atom of the free form.) The resulting conjugated estrogen is no longer of the proper size or chemical character to bind to an estrogen receptor, which is why these forms are biologically inactive.

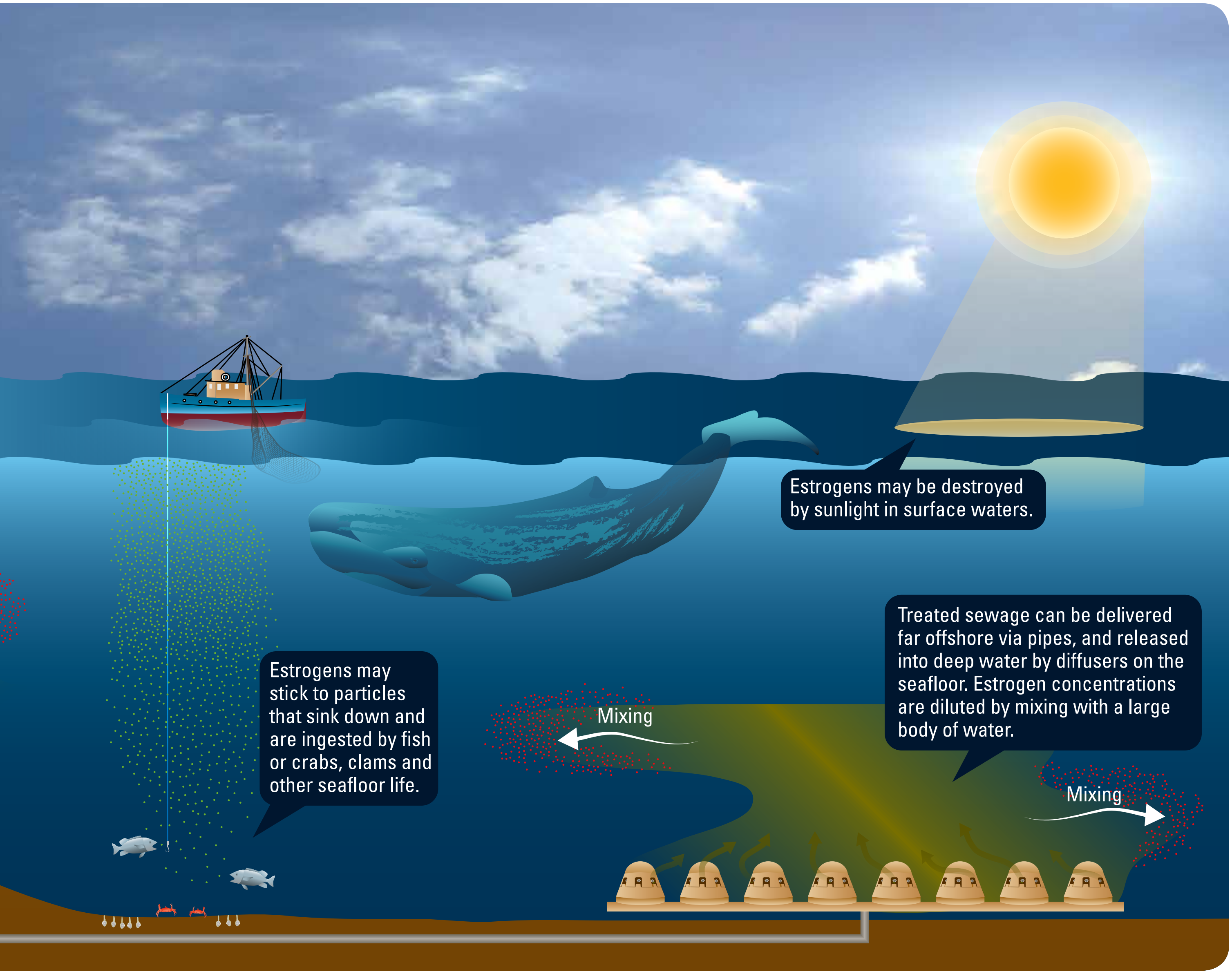
That seems like good news: We are excreting inactive forms. It turns out, though, that bacteria in sewers and sewage treatment plants can convert conjugates back into potent free estrogens.

Sewage treatment alters estrogens in other ways as well. One of the last stages of treatment is disinfection; it is designed to kill harmful germs before effluent is discharged to the environment. Most treatment plants accomplish this by adding what amounts to bleach to the effluent. There is evidence that this step produces chlorinated estrogens that can be nearly as potent as the free forms. What's more, the chemical structure of chlorinated estrogens makes them more likely to accumulate in animal tissues.

A long research row to hoe

Knowing the concentration or "dose" of each form of estrogen—and there might be dozens of them—in Massachusetts Bay is a critical step in determining whether these chemicals might pose a threat to local populations of organisms such as shellfish, flounder, or right whales. If concentrations exceed the "no-harm" threshold set by toxicologists, then it may be desirable to move sewage pipes, alter treatment technologies, or work with pharmaceutical companies to redesign drugs that quickly degrade in treatment plants and receiving waters.

Massachusetts Bay is just one place where understanding the chemical forms



and behaviors of estrogens could lead to insights about how to design cost-effective solutions. The work here can also help us make predictions for many of the other large coastal cities such as Los Angeles, Hong Kong, and Sydney, that release sewage into nearby coastal waters.

But first things first. By dusk, our precious first samples are secure in a small Igloo cooler. As we motor back to Boston in the fading light, I am charting the days of analyses ahead of me. I can't wait to see if there's an estrogen story stuck to the disks in our cooler.

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DAVID **GRIFFITH**

David Griffith grew up in Ann Arbor, Mich., and first became excited about sewage in the summer of 2006 while he was collecting water samples for his master's thesis project from the Hudson River near one of New York City's largest wastewater treatment plants. Huge sewage discharges that contained lots of contaminants and carbon that appeared quite old based on radiocarbon dating got him wondering about the fate of these sewage-derived contaminants in the ocean. Since entering the MIT/WHOI Joint Program, he has used chemical and isotopic methods to understand the flow of carbon and the behavior of estrogens in coastal ocean ecosystems. When he's not extracting seawater, he enjoys biking, angling for trout, and playing upright bass in the bluegrass band Gone To Seed. His mentor on this article was Marguerite Holloway, a contributing editor at *Scientific American* and the director of science and environmental journalism at the Columbia Graduate School of Journalism.